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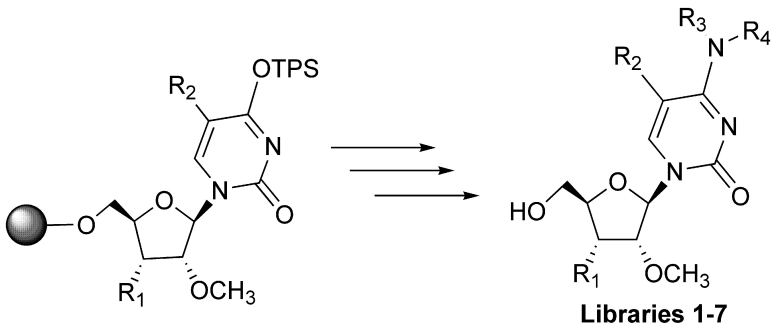

Libraries 1-7

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Synthesis of New 3′-, 5-, and N^4-Modified 2′-*O*-Methylcytidine Libraries on Solid Support

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A versatile solid phase combinatorial approach was developed and utilized for the rapid synthesis of new 2′-*O*-methylcytidine nucleoside libraries **1**−**7** containing 672 compounds with 3′-deoxy-3′-*C*-methyl, 3′-deoxy-3′-*C*-hydroxymethyl, and 5-alkyl/alkynyl modifications. The modified uridine scaffolds **8**−**10**, **23**−**25**, and **31** were loaded onto the 4-methoxytrityl chloride (MMT-Cl) polystyrene resin through the hydroxyl groups at the 5′-position as well as on the substituents at the 3′- and 5-positions. The scaffolds loaded on the resin were orthogonally protected by MMT group on the resin itself and TBDMS or acetyl protecting groups. The 4-position of the uridine derivatives was activated by 2,4,6-triisopropyl benzene sulfonyl chloride for further derivatization. The resins **14**−**16**, **28**−**30**, and **32** loaded with the corresponding activated scaffolds were reacted with the selected and validated amino building blocks in the 96 well format on the semiautomated synthesizer. The high-quality 2′-*O*-methylcytidine libraries **1**−**7** were thus generated and characterized by liquid chromatography−mass spectrometry (LC-MS) analysis with 63−99% successful rates.

Introduction

Nucleoside research has historically been an area of keen interest for small molecule drug discovery due predominately to the medicinal value and biological importance of these molecules. Among various nucleoside analogues, uridine and cytidine nucleoside analogues have shown significant antiviral and antineoplastic activity.[1] Various antiviral activities of 5-substituted uridine derivatives have been reported.[2] Different *N*-substituted cytidines have shown biological activity and therapeutic applications, such as 2′,3′-dideoxycytidine (Zalcitabine),[3] which is one of the nucleoside drugs clinically used. Unfortunately, many known nucleoside analogues that inhibit tumor growth or viral infections are also toxic to normal mammalian cells primarily because these nucleoside derivatives lack adequate selectivity between the normal cells and the virus-infected host cells or cancers cells. It was reported that 2′-*O*-modified oligonucleotides improved biochemical properties such as enhancing RNase H binding affinity.[4] Several oligonucleotides with 2′-*O*-mehtyl modifications are currently in clinical trials including the antiangiogenic ribozyme molecule, RPI 4610.[5] However, antiviral and anticancer drug discovery studies of new cytidine derivatives have not been well-explored. Therapeutic application of some nucleoside analogues stimulated our research interest to design and rapidly synthesize new 2′-*O*-methyl cytidine derivatives with various modifications at 3′-, 5-, and N^4-positions to discover new antiviral and anticancer agents.

A variety of solid phase[6] and solution phase[7] combinatorial approaches have been used for the generation of different libraries for a wide range of biological screening. However, these combinatorial approaches have not been well-utilized for the rapid synthesis of novel nucleoside libraries. Different purine and pyrimidine nucleoside derivatives were synthesized in solution by traditional approaches[8] because of the tremendous difficulties to apply solid phase combinatorial strategies to nucleoside chemistry. We recently reported the synthesis of novel exocyclic amino ribofuranosyl nucleoside libraries by a newly developed parallel solid phase combinatorial approach.[9] To rapidly produce a large number of diverse nucleosides for our drug discovery programs, we utilized the semiautomated parallel solid phase approach for the synthesis of new cytidine libraries.

Herein, we report the rapid synthesis of new 2′-*O*-methylcytidine nucleoside libraries **1**−**7** (672 compounds) with 3′-deoxy-3′-*C*-methyl, 3′-deoxy-3′-*C*-hydroxymethyl, and 5-alkyl/alkynyl modifications (Figure 1). The 4-positions

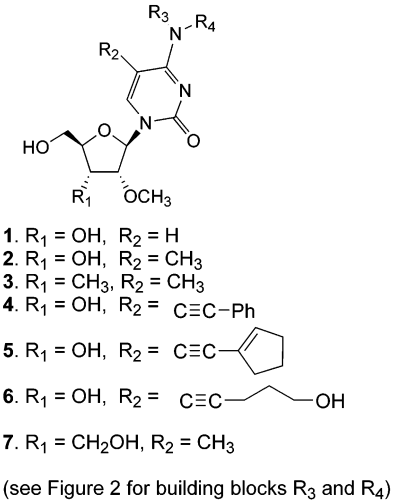

Figure 1. New 2′-*O*-methyl cytidine libraries **1**−**7**.

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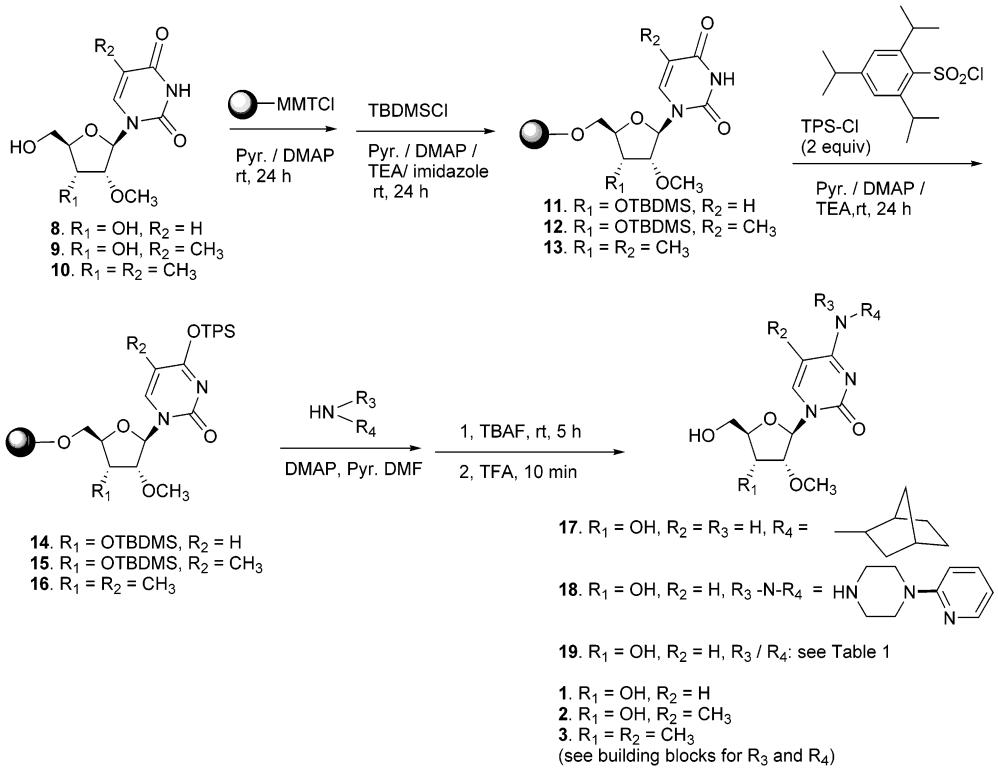
Scheme 1. Development and Synthesis of 2′-*O*-Methyl Cytidine Libraries **1**−**3**



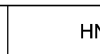
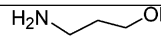
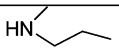
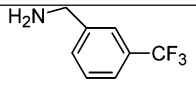
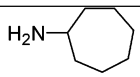
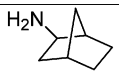
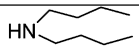
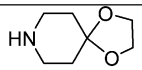
of these scaffolds were combinatorialized by 96 selected and validated amino building blocks. New substituted 2′-*O*-methyluridine derivatives **21**−**25** were synthesized as scaffolds. The modified uridine scaffolds **8**−**10**, **23**−**25**, and **31** were loaded onto the 4-methoxytrityl chloride (MMT-Cl) polystyrene resin through the hydroxyl groups at the 5′-, 3′-, and 5-positions. The 4-position of the uridine derivatives on resins was activated by its reaction with 2,4,6-triisopropyl benzene sulfonyl chloride (TBS-Cl). Resins **14**−**16**, **28**−**30**, and **32** loaded with the activated uridine scaffolds were reacted with the selected amino building blocks in the 96 well format on the semiautomated synthesizer to generate high-quality libraries **1**−**7** with 63−99% successful rate.[10]

Results and Discussion

Although hundreds of purine and pyrimidine nucleosides were synthesized for a variety of biological and biomedical stuides,[8] only a handful of 2′-*O*-methylribofuranosyl cytidine derivatives with *N*-methyl, *N*,*N*-dimethyl,[11] 5-methyl,[12] and 5-propynyl[13] modifications were reported. To thoroughly explore the biological and biomedical properties of 2′-*O*-methylcytidine derivatives, we designed and synthesized new 3′-deoxy-3′-*C*-methyl, 3′-deoxy-3′-*C*-hydroxymethyl, and 5-alkyl/alkynyl substituted 2′-*O*-methylcytidine derivatives by combinatorializing the 4-position with a variety of amino building blocks.

Scheme 1 outlines the development and validation of the parallel solid phase strategy as well as the synthesis of new libraries **1**−**3**. 2′-*O*-Methyluridine **8** was reacted with MMT-Cl polystyrene resin in anhydrous pyridine at room temperature in the presence of 4-(*N*,*N*-dimethyl)pyridine (DMAP). Scaffold **8** was attached onto the resin through the 5′-hydroxy group, which is more reactive than the 3′-hydroxyl group. The resin that resulted was treated with an excess amount of *tert*-butyldimethylsilyl chloride to protect the 3′-hydroxyl group. The MMT resin acted as a solid support for the solid phase synthesis and as a protecting group to block the 5′-hydroxyl group for the orthogonal protection together with other protecting groups. The MMT protecting group on the resin was easily removed by mild acidic conditions to release the final product. Therefore, our current and previous reported research[9] indicated that the MMT-Cl polystyrene resin is an efficient solid support for the combinatorial nucleoside chemistry and can be widely utilized for the synthesis of nucleoside libraries. The resultant protected uridine resin **11** was reacted with TBS-Cl in the presence of DMAP and triethylamine in pyridine to activate the 4-position of the uridine scaffold. Thus obtained resin **14** was confirmed by MAS NMR analysis (see Supporting Information). The TBS moiety on resin **14** is an excellent leaving group, and it can be easily substituted by a variety of nucleophiles, such as amines, to give the corresponding cytidine type of derivatives. To generate a high-quality library from resin **14**, we first manually validated resin **14** utilizing *N*-(pyridin-2-yl)piperazine having reduced nucleophilicity and sterically hindered *exo*-2-aminonorborane as nucleophiles. Resin **14** was shaken with these two selected amines in parallel at room temperature for 24 h in the presence of DMAP. The resins that resulted were treated with tetrabutylammonium fluoride (TBAF) to deprotect the TBDMS protecting group at the 3′-position. The resin needs to be thoroughly washed at this stage to completely remove TBAF salt and to ensure the quality of final product. The dried resins were treated with 2% trifluoroacetic acid (TFA) in 1,2-dichloroethane to cleave the products off the resins. The products **17** and **18** obtained directly from resins were

Table 1. Validation Results for Resin **14** on Synthesizer

Entry		LC-MS purity of **19**
a		100%
b		100%
c		70%
d		100%
e		90%
f		100%
g		100%
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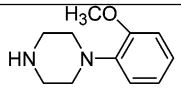
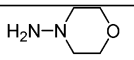
i		90%
j		-

analyzed by liquid chromatography−mass spectrometry (LC-MS) without purification. Compounds **17** and **18** were characterized by ES mass spectrometry, and showed 93.4 and 95.6% LC purity, respectively. Compound **18** was also verified by NMR spectral analysis as a representative example. Six consecutive steps for the synthesis of these two compounds on solid support are required. Such a high purity for the final products indicated that the yield for each step had to be more than 99%. These results also indicated very high efficiency for the synthesis of nucleosides on solid support.

After manually and successfully validating resin **14**, we further validated the different types of amino building blocks on synthesizer utilizing the similar reaction conditions and procedures. Resin **14** was reacted in parallel with different amine-types of nucleophiles listed in Table 1 under the same conditions described above. After deprotection and cleavage, the products **19a**−**j** were dried and analyzed by LC-MS without purification. Table 1 shows the LC-MS purity of these products. The results indicated that the primary (entry **a**) and secondary (entries **b** and **f**−**i**) as well as steric hindered (entry **e**) amines all gave the desired products with high purities of 90−100%. The electron-withdrawing group CF_3 on the benzene ring reduces the nucleophilicity of the benzylamine; therefore, the corresponding product **19c** was obtained with only 70% purity (entry **c**). The hydrazine type of nucleophile (entry **j**) did not generate the desired product. At the same time, we also synthesized one plate of 96 compounds, library **1**, on Vanguard synthesizer. Resin **14** was reacted with 96 primary/secondary amino, hydrazino, and hydroxyamino building blocks in group I (Figure 2) and group II (Figure 3; see Supporting Information). After deprotection and cleavage under the conditions as described above, 96 wells of samples were obtained and directly analyzed by LC-MS. The results indicated that 75% of the 96 samples in library **1** showed 60−100% purity (Table 2). Some α,ω-diamines with two reactive sites, hydrazine type, and other building blocks in group II (Supporting Information) did not give the desired products in high yields. Therefore, we excluded these nonqualified building blocks and added 46 more amino building blocks in group III (Figure 2) for further library synthesis.

Table 2. Quality of Libraries **1**−**7**[a]

library no.	percentage of products >60%[a]	library no.	percentage of products >60%[a]
1	75	**5**	90
2	99	**6**	63
3	84	**7**	98
4	88	overall	85.3

[a] Ninety-six compounds for each library.

The strategy and reaction conditions for the preparation of resin **14** were utilized for the preparation of resin **15** from the corresponding 5-methylribofuranosyl uracil **9**. The resin **16** loaded with 3′-deoxy-3′-*C*-methyl-5-methyl pyrimidine nucleoside scaffold was obtained by the same strategy from 3′-deoxy-3′-*C*-methyl-5-methylribofuranosyl uracil **10**[14,15] except that the TBDMS protection was not required. Resins **15** and **16** were reacted in a parallel fashion with 96 selected building blocks in groups I and III (Figure 2) on synthesizer by the same procedures as described above. After deprotection and cleavage, libraries **2** and **3** were obtained, and each of them contains 96 compounds. Direct LC-MS analysis indicated that the successful rates for libraries **2** and **3** are 99 and 84%, respectively. Ninety-eight out of 99 compounds in library **2** showed more than 60% LC purity. This indicated that the highest quality for nucleoside libraries was synthesized on solid support.

Schemes 2 and 3 outline the synthesis of libraries **4**−**6** with 5-alkynyl modifications. 2′-*O*-Methyl-3′,5′-*O*-diacetyl-5-iodoribofuranosyl uracil **20** was synthesized based on the reported procedures from compound **8**.[16] 5-Iodoridine derivative **20** was coupled with different substituted alkynes under Heck reaction conditions. Tris(dibenzylideneacetone) dipalladium(0) [$Pd_2(dba)_3$] was used as the catalyst for the C−C bond formation at the 5-position. New 5-alkynylated uridine derivatives **21**−**23** were obtained in 62−75% yields and characterized. Compounds **21** and **22** were deprotected by treating with a saturated ammonia solution in methanol providing the corresponding new uridine scaffolds **24** and **25** in high yields. Compounds **24** and **25** with free hydroxyl group at 5′-position were ready to be attached onto solid support. Compound **23** had a hydroxyl group on the 5-substituent for its attachment onto solid support; therefore, the deprotection step was not required. Compounds **24** and **25** were reacted with MMT-Cl polystyrene resin in anhydrous pyridine under the same condition as described above for the preparation of resins **11**−**13**. The resulting resins **26** and **27** were acylated by acetic anhydride to protect the 3′-hydroxyl group. The 4-position of uridines was activated by TBS-Cl to give the loaded resins **28** and **29**, which were

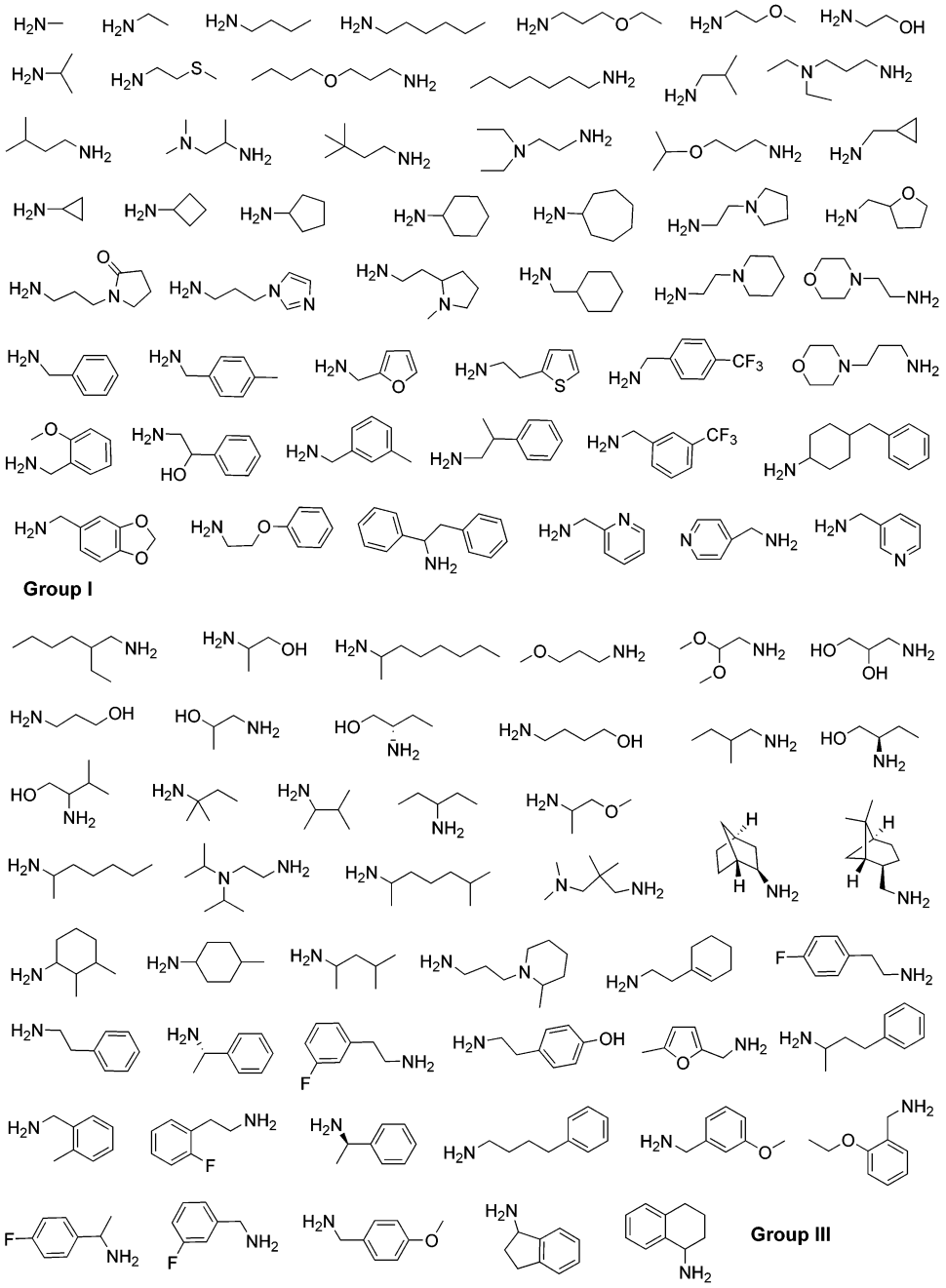
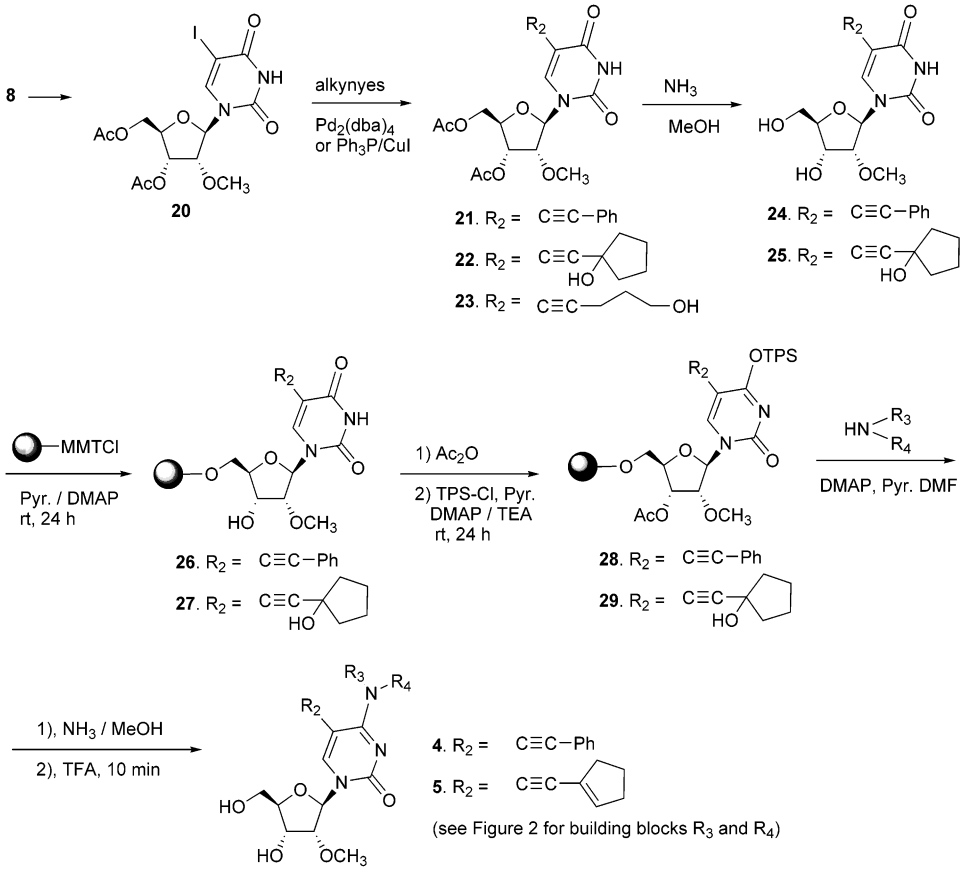


Figure 2. Building blocks for library synthesis.

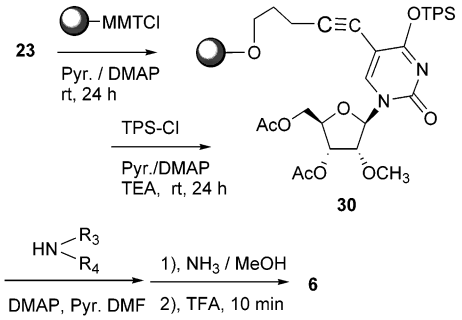
ready for the automated parallel synthesis of libraries **4** and **5**. As described above for the synthesis of libraries **1**−**3**, resins **28** and **29** were reacted in a parallel fashion with 96 selected amino building blocks in groups I and III (Figure 2) on the Vanguard 96 well synthesizer. The resulting resins were deprotected with a saturated ammonia solution in methanol. The products were cleaved from solid support with 1% TFA solution in 1,2-dichloroethane. Library **4** was obtained with an 88% successful rate based on LC-MS analysis. The heating reaction condition for the nucleophilic substitution caused the elimination of the hydroxyl group next to the triple bond and cyclopantanyl group. Therefore, the eliminated product library **5** with the conjugated cyclic pentenylethynyl substituent at position 5 was obtained with the success rate of 90% instead of the products with hydroxyl cyclic pentanyl ethynyl substituents at position 5. The 3′,5′-diacylated uridine derivative **23**, having a hydroxyl group on the 5-substituent, was directly loaded onto the MMT-Cl resin through the existing hydroxyl group (Scheme 3). After it was activated with TBS-Cl, the resultant resin **30** was reacted in parallel with 96 amino building blocks in groups I and III (Figure 2) on synthesizer as described above. Deprotection and cleavage under the same reaction conditions as described provided library **6** with a 63% successful rate.

5′-*tert*-Butyldiphenylsilyl-3′-deoxy-3′-*C*-hydroxymethyl-2′-*O*-methyl-5-methyluridine (**31**) was synthesized based on the reported procedures.[14,15] Compound **31** was reacted with MMT-Cl resin to load the scaffold onto MMT resin through the available hydroxyl group at the 3′-position. Thus, obtained resin was activated by TBS-Cl under the same condition as described above to give resin **32** with TBS as the good leaving group. Resin **32** was reacted in parallel with

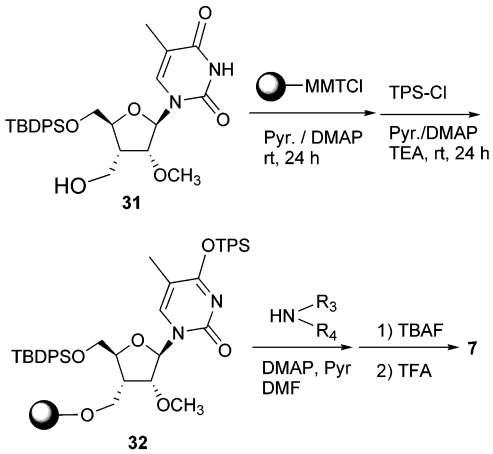
Scheme 2. Synthesis of 5-Substituted 2′-*O*-Methyl Cytidine Scaffolds and Libraries **4** and **5**



Scheme 3. Synthesis of Cytidine Library **6** by Attaching the 5-Position on Solid Support



Scheme 4. Synthesis of 2′-*O*-Methyl Cytidine Library **7** by Attaching the 3′-Position on Solid Support



96 amino building blocks in groups I and III (Figure 2). The resulting resins were deprotected with TBAF and then cleaved by TFA under similar conditions as described above for the synthesis of libraries **1**−**3**. High-quality library **7** was obtained with a 98% success rate, which indicated that 95 desired compounds out of 96 synthesized samples showed 60−100% LC purity.

In conclusion, we have successfully utilized the parallel solid phase combinatorial approach for the rapid synthesis of new pyrimidine nucleoside libraries. MMT-Cl polystyrene resin was approved to be an efficient solid support for nucleoside library synthesis. Hydroxyl groups at the 5′-postion as well as on the substituents at the 3′- and 5-positions were effectively utilized to attach the nucleoside scaffolds onto the MMT-Cl polystyrene resin. The TBS group is an excellent leaving group for various nucleophilic substitutions on the pyrimidine base to synthesize high-quality nucleoside libraries. Libraries **1**−**7**, containing 672 new 2′-*O*-methylcytidine nucleosides with 3′-, 5-, and N^4-modifications, were synthesized by the parallel solid phase combinatorial approach with an overall success rate of 85.3%. New 2′-*O*-methylcytidine derivatives (571) were registered into the database, and the 101 less pure compounds were purified on the parallel purification system, Biotage Parallex Flex HPLC. The parallel solid phase combinatorial approach can be widely utilized for the synthesis of other pyrimidine and purine nucleoside libraries. These new 2′-*O*-methylcytidine derivatives with various modifications at the 3′-, 5-, and N^4-positions are being screened against a wide range of biological assays.

Experimental Section

General Methods. NMR spectra were recorded at 300 MHz, and the chemical shifts were expressed relative to the added tetramethylsilane. The libraries were enumerated by Afferent TeamWorks 3.0, labeled and weighted by Label Automador, and synthesized on the ACT Vanguard semi-automated synthesizer. Libraries were analyzed on a LC-MS system. The LC-MS system consisted of a Waters 2790 HPLC, Waters 996 photodiode array (PDA) detector, and Micromass/Waters ZQ mass spectrometer. A 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 absorbency at 220 nm. The LC-MS operation was controlled by MassLynx software, and the LC-MS data were processed by OpenLynx software. MMT-Cl polystyrene resin was purchased from Novabiochem. Other starting materials, building blocks, and reagents were purchased from Aldrich and other companies and used directly. 3′-Dexoy-3′-*C*-methyl-2′-*O*-methyl-5-methyluridine (**10**)[14,15] and 5′-*O*-(*tert*-butyldiphenylsilyl)-3′-deoxy-3′-*C*-hydroxymethyl-2′-*O*-methyl-5-methyluridine (**31**)[15] were prepared according to the reported procedures. 3′,5′-*O*-Diacetyl-2′-*O*-methyl-5-iodoridine (**30**)[16] was prepared from 2′-*O*-methyluridine (**8**).

General Procedures for the Preparation of 5′-(4-Methyoxytrityl Polystyrene) Resins 14−16. A suspension of 4-methoxytrityl chloride polystyrene resin (4.05 g, 7.0 mmol; Novabiochem, loading capacity ∼1.7 mmol/g), 2′-*O*-methyluridine derivatives **8**, **9**, or **10** (8.4 mmol, 1.2 equiv), and DMAP (100 mg) in 25 mL of anhydrous pyridine was shaken at room temperature for 24 h. The resin was filtered and washed sequentially with pyridine−DMF (1:1, v/v, four times) and then with dichloromethane (three times). The thus obtained resin was dried under vacuum and suspended in a mixture of pyridine (20 mL), dichloromethane (10 mL), and triethylamine (3.0 mL). *tert*-Butyldimethylsilyl chloride (TBDMS-Cl) (5.27 g, 35 mmol, 5 equiv) and imidazole (2.38 g, 34.5 mmol) were added followed by the addition of DMF (5 mL) to improve the solubility and swelling ability. The resultant suspension was shaken at room temperature for 24 h. The resin was washed sequentially with pyridine−DMF (1:1, v/v, four times) and dichloromethane (four times) and then dried under vacuum. The thus obtained resin **11**, **12**, or **13** and DMAP (100 mg) were suspended in a mixture of dichloromethane (30 mL) and triethylamine (6.8 mL). After the suspension was shaken for 30 min, TBS-Cl (4.24 g, 14 mmol, 2 equiv) was added. The resulting reaction mixture was shaken at room temperature for 24 h. Methanol (2 mL) was added to the reaction mixture to destroy the excess amount of TBS-Cl. The resin was washed sequentially with pyridine−DMF (1:1, v/v, five times, containing a trace amount of water for the first two washes) and dichloromethane (three times). A 7−8 g amount of resin **14**, **15**, or **16** was obtained after dried under vacuum.

2′-*O*-Methyl-N^4-(*exo*-norboran-2-yl)cytidine (17) (Validation of Resin 14). A suspension of resin **14** (100 mg), *exo*-2-aminonorborane (118 μL, 110 mg, 1.0 mmol), DMAP (40 mg), and *N*,*N*-diisopropylethylamine (100 μL) in a mixture of anhydrous DMF (1.5 mL) and pyridine (1.5 mL) was shaken at room temperature overnight. The resin was filtered and washed sequentially with pyridine (three times), DMF (three times), and dichloromethane (three times). The resin was dried and suspended in 3 mL of DMF. Tetrabutylammonium fluoride (TBAF, 1 M, 1 mL) was added. After it was shaken at room temperature for 5 h, the resin was filtered and washed thoroughly with pyridine (four times), DMF (four times), and then dichloromethane (four times). The dried resin was swelled in 4 mL of 1,2-dichloroethane, and the suspension was treated dropwise with 80 μL of TFA with gentle shaking. The typical red-orange color appeared immediately and disappeared upon adding 1 mL of methanol after 10 min. The resin was filtered and washed three times with methanol. The filtrates were collected and concentrated to dryness to provide 55 mg of compound **17** as a pale yellow foam. The product showed 93.4% LC purity. ES MS *m*/*z* 352 $(M + 1)^+$.

2′-*O*-Methyl-N^4-(*N*-pyridin-2-yl)piperazinylcytidine (18). Compound **18** was prepared as described above for the preparation of compound **17** from 100 mg of resin **14** and *N*-(pyridin-2-yl)piperazine. Sixty milligrams of **18** was obtained as a pale yellow foam in 95.6% LC purity. ^{1}H NMR (CD_3OD): δ 3.55 (s, 3H), 3.74−3.82 (m, 1H), 3.84−3.90 (m, 1H), 3.95−4.18 (m, 6H), 4.25 (d, 1H, $J = 4.8$ Hz), 5.94 (d, 1H, $J = 1.6$ Hz), 6.42 (d, 1H, $J = 5.4$ Hz), 7.06 (t, 1H, $J = 4.6$ Hz), 7.36 (d, 1H, $J = 6.4$ Hz), 8.03 (d, 1H, $J = 4.2$ Hz), 8.05−8.14 (m, 1H), 8.58 (d, 1H, $J = 5.4$ Hz). ES MS *m*/*z* 404 $(M + 1)^+$, 807 $(2M + 1)^+$.

Synthesis of Compounds 19a−j (Validation of Resin 14 on Synthesizer). Amine Substitution. Approximately 30 mg of starting resin **14** was dispensed in 10 reaction wells using a dispensing spatula and funnel. To each well of resin were added 100 μL of anhydrous DMF, 0.5 mL (1 M) of the appropriate amine (see Table 1) in DMF, and then 0.5 mL of DMAP/DIEA solution in anhydrous pyridine (pyridine, 15 mL; DMAP, 400 mg; DIEA, 1 mL). The reaction block was covered and shaken at room temperature for 14 h. The reaction vials were emptied and washed sequentially with DMF (three times), MeOH/H_2O (6:4, three times), MeOH/dichloromethane (1:1, three times), and finally with dichloromethane (two times). The resultant resins were dried under nitrogen.

Deprotection. To the dried resin in each well was added 1 mL of anhydrous DMF followed by 0.5 mL of TBAF (1 M) solution in THF. The reaction mixtures were shaken at room temperature for 6 h. The wells were emptied and then washed with DMF (three times), MeOH/dichloromethane (1:1, three times), and finally with dichloromethane (two times). The resultant resins were dried under nitrogen.

Cleavage. TFA (2% in dichloromethane) was added to the resin in each well. The reaction mixtures were shaken at room temperature for 7 min. The solutions were filtered into the prelabeled and preweighed vials. The resins were washed with MeOH (1 mL) and filtered into the same corresponding vials. To the combined filtrates was added toluene (0.25 mL).

The solvents were evaporated under vacuum using Savant SpeedVac Plus vacuum evaporator to provide compounds **19a**−**j** (Table 1).

General Procedure for the Synthesis of 3′,5′-Di-*O*-acetyl-2′-*O*-methyl-5-(substituted acetylenyl)uridines (21−**23).** To a stirred solution of 3′,5′-di-*O*-acetyl-5-iodo-2′-*O*-methyluridine (**20**)[16b] (0.47 g, 10.0 mmol) in 10 mL of anhydrous DMF were added triethylamine (1 mL), the corresponding substituted acetylene derivatives (20 mmol), tris(dibenzylideneacetone)−dipalladium(0) (20 mg), copper-(I) iodide (10 mg), and triphenylphosphine (100 mg). The reaction mixture was stirred at 35 °C under a nitrogen atmosphere for 18 h. The resultant reaction mixture was concentrated to dryness under vacuum. The resultant residue was purified by flash chromatography on a silica gel column using hexanes−ethyl acetate (1:1) as an eluent to provide the products **21**−**23**.

3′,5′-Di-*O*-acetyl-2′-*O*-methyl-5-phenylacetylenyluridine (21). Compound **21** was synthesized as a white foam in 75% yield (0.33 g) from compound **20** and phenylacetylene as described above. ^{1}H NMR ($CDCl_3$): δ 2.14 (s, 3H), 2.20 (s, 3H), 3.67 (s, 3H), 4.19 (d, 1H, J = 4.8 Hz), 4.50 (m, 3H), 4.81 (q, 1H, J = 5.1 Hz), 6.09 (s, 1H), 6.68 (s, 1H), 7.41 (3H, m), 7.29 (d, 2H), 8.48 (s, 1H). HRMS *m*/*z* 443.145 $(M + H)^+$ ($C_{22}H_{23}N_2O_8$ requires 443.145).

3′,5′-Di-*O*-acetyl-5-[(1′-hydroxycyclopentanyl)acetylenyl]-2′-*O*-methyluridine (22). The title compound was synthesized as a white foam in 65% yield from compound **20** and 1-ethynylcyclopentanol as described above. ^{1}H NMR ($CDCl_3$): δ 2.13 (s, 3H), 2.21 (s, 3H), 3.48 (s, 3H), 4.05 (q, 1H, J = 2.4 Hz), 4.37 (m, 3H), 4.94 (t, 1H, J = 4.8 Hz), 5.92 (d, 1H, J = 4.8 Hz), 7.83 (s, 1H), 10.17 (s, 1H). HRMS *m*/*z* 451.171 $(M + H)^+$ ($C_{21}H_{27}N_2O_9$ requires 451.170).

3′,5′-Di-*O*-acetyl-5-(5-hydroxypentyn-1-yl)-2′-*O*-methyluridine (23). The title compound was synthesized as a white foam in 62% yield from compound **20** and 4-pentyn-1-ol as described above. ^{1}H NMR (CD_3OD): δ 1.77 (m, 2H), 2.13 (s, 3H), 2.19 (s, 3H), 2.48 (t, 2H, J = 6.0 Hz), 3.45 (s, 3H), 3.66 (t, 2H, J = 6.0 Hz), 4.15 (dd, 1H), 4.37 (m, 4H), 5.14 (m, 1H), 5.90 (d, 1H, J = 3.3 Hz), 7.94 (s, 1H). HRMS *m*/*z* 425.156 $(M + H)^+$ ($C_{19}H_{25}N_2O_9$ requires 425.155).

5-Phenylacetylenyl-2′-*O*-methyluridine (24). Compound **21** (15 mg, 0.034 mmol**)** was dissolved in 4 mL of MeOH saturated with NH_3. The reaction mixture was sealed, stirred at room temperature overnight, and then concentrated under reduced pressure. The resultant residue was purified by flash chromatography on a silica gel column using CH_2Cl_2−MeOH (10:1) as an eluent to afford 11 mg of product **24** as a white solid in 90% yield. ^{1}H NMR (CD_3OD): δ 3.59 (s, 3H), 3.80 (d, 1H, J = 12.6 Hz), 3.95 (m, 1H), 4.00 (d, 1H, J = 12.6 Hz), 4.01 (m, 1H), 4.50 (t, 1H), 5.95 (d, 1H, J = 3.6 Hz), 7.36 (m, 3H), 7.55 (m, 2H), 8.60 (s, 1H). HRMS *m*/*z* 359.124 $(M + H)^+$ ($C_{18}H_{19}N_2O_6$ requires 359.123).

5-[(1′-Hydroxycyclopantanyl)acetylenyl]-2′-*O*-methyluridine (25). The title compound was synthesized as a white solid in 85% yield from compound **22** as described above for the preparation of compound **24**. ^{1}H NMR (CD_3OD): δ 1.79 (m, 4H), 1.96 (m, 4H), 3.59 (s, 3H), 3.76 (dd, 1H, J = 12.6, 2.7 Hz), 3.85 (q, 1H), 3.91 (dd, 1H, J = 12.6, 2.7 Hz), 4.25 (t, 1H, J = 6.3 Hz), 5.92 (d, 1H, J = 3.0 Hz), 8.42 (s, 1H). HRMS *m*/*z* 367.150 $(M + H)^+$ ($C_{17}H_{23}N_2O_7$ requires 367.149).

General Procedure for the Preparation of 3′-*O*-Acetyl-5-(substituted acetylenyl)-2′-*O*-methyl-4-(2,4,6-triisopropylbenzenesulfonyl)uridine-5′-*O*-(4-methoxytrityl polystyrene)resins 28 and 29. To a solution of 5-(substituted acetylenyl)-2′-*O*-methyluridine derivative **24** or **25** (5 mmol) in anhydrous pyridine (40 mL) were added 4-methoxytrityl chloride polystyrene resin (4.0 g) and DMAP (200 mg). The suspension was shaken at room temperature for 48 h. Methanol (2 mL) was added. After shaking for an additional 30 min, the resulting resin **26** or **27** was filtered, washed sequentially with pyridine (three times), DMF (three times), and then dichloromethane (three times). The resin **26** or **27** was dried and suspended in pyridine (20 mL). Acetic anhydride (15 mL) was added, and the suspension was shaken at room temperature for 10 h. The resulting resin was filtered, washed as described above, and dried. The thus obtained resin was suspended in a mixture of DMAP (150 mg) and triethylamine (6 mL) in dichloromethane (40 mL). After shaking at room temperature for 1 h, TBS-Cl (4.5 g) was added. The suspension continued to be shaken at room temperature for an additional 6 h. Methanol (2 mL) was added to destroy the excess amount of TBS-Cl. The resulting resin **28** or **29** was filtered, washed as described above for resins **14**−**16**, and dried.

3′,5′-Di-*O*-acetyl-2′-*O*-methyl-4-(2,4,6-triisopropylbenzenesulfonyl)-5-(5-*O*-MMT polystryrene-hydroxypentyn-1-yl)uridine Resin 30. To a solution of compound **23** (5 mmol) in anhydrous pyridine (40 mL) were added MMT-Cl polystyrene resin (4.0 g) and DMAP (200 mg). The suspension was shaken at room temperature for 48 h. Methanol (2 mL) was added. After shaking for an additional 30 min, the resulting resin was filtered, washed with pyridine (three times), DMF (three times), and dichloromethane (three times). The resin was dried and then suspended in a mixture of DMAP (150 mg) and triethylamine (6 mL) in dichloromethane (40 mL). After shaking at room temperature for 1 h, TBS-Cl (4.5 g) was added. The reaction mixture continued to be shaken at room temperature for 6 h. Methanol (2 mL) was added to destroy the excess amount of TBS-Cl. The resulting resin **30** was filtered, washed as described above for resins **14**−**16**, and dried.

5′-*O*-(*t*-Butyldiphenylsilyl)-3′-deoxy-3′-*C*-hydroxymethyl-2′-*O*-methyl-5-methyl-4-(2,4,6-triisopropylbenzenesulfonyl)uridine 3′-*O*-MMT-methylenepolystyrene Resin (32). A suspension of 4-methoxytrityl chloride polystyrene resin (4.7 g, 8.0 mmol), 5′-*O*-(*tert*-butyldiphenylsilyl)-3′-deoxy-3′-*C*-hydroxymethyl-2′-*O*-methyl-5-methyluridine (**31**)[15] (4.8 g, 9.1 mmol), and DMAP (150 mg) in 35 mL of anhydrous pyridine was shaken at room temperature for 24 h. The resin was filtered and washed with pyridine−DMF (1:1, four times) and then dichloromethane (four times). A suspension of the dried resin and DMAP (200 mg) in dichloromethane (40 mL) containing triethylamine (10 mL) was shaken for

30 min. 2,4,6-Triisopropyl benzene sulfonyl chloride (6.0 g, 19.8 mmol) was added. The resulting suspension was shaken at room temperature for 24 h. Methanol (2 mL) was added to the reaction mixture. The resin was washed sequentially with pyridine−DMF (1:1, five times) and then with dichloromethane (three times). A 9.53 g amount of red orange resin **32** was obtained after it was dried under vacuum.

General Procedures for the Parallel Synthesis of Libraries 1−3 and 7. Amine Substitution. Approximately 70−75 mg of starting resin **14**−**16** or **32** was dispensed in each of the 96 reaction wells using a dispensing spatula and funnel. To each well of resin were added 100 μL of DMF (or NMP), 0.5 mL (1 M) of appropriate amines in DMF (or NMP), and then 1.0 mL of DMAP/DIEA solution in anhydrous pyridine (pyridine, 100 mL; DMAP, 4.0 g; DIEA, 10.0 mL). The reaction block was covered and shaken at room temperature overnight. The wells were emptied, and the resins were washed with DMF (three times), MeOH/dichloromethane (1:1, three times), and finally with dichloromethane (two times). The resins were then dried under nitrogen.

Silyl Deprotection. Tetrabutylammonium fluoride (1.0 M in THF, 1.5 mL) was added to each well of resins, and the reaction mixtures were shaken at room temperature overnight (∼16 h). After emptying and washing with DMF, the resin was washed three times with 10% acetic acid in DMF and then washed as usual with DMF (three times), MeOH/dichloromethane (1:1, three times), and finally with dichloromethane (two times). The resulting resins were dried under nitrogen.

Cleavage. TFA (1.5% in 1,2-dichloroethane) was added to the resin in each well. The reaction mixtures were shaken for 2 min. The solutions were filtered into the prelabeled and preweighed vials. The resins were washed with MeOH (1 mL) and filtered into the same corresponding vials. To the combined filtrates was added toluene (0.25 mL), and the solvent was evaporated in vacuo using Savant SpeedVac Plus vacuum evaporator to provide final libraries.

General Procedures for the Parallel Synthesis of Libraries 4−6. Libraries **4**−**6** were synthesized from resins **28**, **29**, or **30** by the similar procedures as described above for libraries **1**−**3** except using the deprotection procedure as follows: To the resins was added 1.5 mL of methylamine (2.0 M) solution in methanol. The plate was covered with top plate (airtight) and shaken at room temperature overnight. After the plate was emptied, the resins were washed with DMF (three times), MeOH/dichloromethane (three times), and finally with dichloromethane (two times). The resins were then dried under nitrogen.

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Supporting Information Available. MAS NMR of resin **14**, LC-MS profiles of 10 representative library members, building block group II (Figure 3), structural and quality data of the representative library **7** (Table 3), and the NMR data of the representative library members (Table 4). This material is available free of charge via the Internet at http://pubs.acs.org.

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