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An efficient PyAOP-based C^4-amination method for direct access of oxidized ^{5Me}dC derivatives

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ABSTRACT

In the past decade, synthetic oxidized $^{5\text{-Me}}$dC nucleosides and their derivatives have become essential tools for epigenetic research. The low efficacy of both conventional and newly reported BOP methods on C^4-amination of these specific oxidized $^{5\text{-Me}}$dU substrates urged us to systematically investigate how the nature of onium salt-based coupling reagents affects the C^4-amination of pyrimidine nucleobases and lead us to the findings that different onium coupling reagents result in the formation of distinctive activation intermediates and PyAOP is much more potent than BOP in both activation and aminolysis steps. Direct amination without the need of ribose protection, ultrafast activation, tolerance to aqueous *N*-nucleophiles, and excellent yields for diverse oxidized ^{5Me}dC derivatives are the advantages of this PyAOP-based C^4-amination method.

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1. Introduction

In mammalian DNA, cytosines are predominantly methylated within CpG sites, and the methylation patterns could be passed on to the next generation as stable epigenetic signals during cell divisions [1]. In the past 20 years, it has been revealed that DNA methyltransferases-mediated cytosine methylation in eukaryotic DNA is one of the most crucial epigenetic marks for transcriptional gene silencing [2]. Meanwhile, DNA demethylation is also highly important for the recovery of cytosine, thereby rendering flexible regulation of gene expression during cellular development [3]. A series of oxidized 5-methylcytosine (5-mC) derivatives, such as 5-hydroxymethylcytosine (5-hmC), 5-formyl-cytosine (5-fC), and 5-carboxycytosine (5-caC) have been isolated from mammalian DNA [4–6]. The evidences reported by He [7] and Zhang [8] groups strongly indicated that ten-eleven translocation proteins (TET)-mediated oxidation of 5-mC leads to active DNA demethylation in epigenetic reprogramming of cells [9,10].

To facilitate the investigation of mechanisms and enzymes involved in 5-mC oxidation, solid phase approaches for the preparation of 5-hmC-, 5-fC-, and 5-caC-containing oligodeoxynucleotides (ODNs) have been developed [11–13]. Later, Carell and co-workers achieved expeditious synthesis of long 5-hmC-, 5-fC-, and 5-caC-containing ODNs from ^{5HOMe}-, $^{5\text{-CHO}}$-, and $^{5\text{-COOH}}$-dC triphosphates by polymerase chain reaction [14]. Apparently, the chemical synthesis of various oxidized ^{5Me}dC nucleosides, phosphoramidite monomers, and nucleoside triphosphates plays a crucial role in the progress of epigenetic research.

Our ongoing research on the synthesis of oxidized ^{5Me}dC derivatives showed that C^4-amination of the corresponding oxidized ^{5Me}dU precursors is one of the most challenging steps. As we described in previous reports [15,16], the conventional $POCl_3$/1,2,4-triazole/conc. NH_4OH [17,18] or TsCl/1-methylpiperidine/conc. NH_4OH [19] methods could afforded the desired oxidized 3′,5′-*O*-diTBS-protected 5^{Me}dC derivatives in 60–70% yields. However, due to the reactivity of the activating reagents to OH groups, ribose moiety must be fully protected before C^4-amination. In addition, hydrolysis of the triazole or quaternary ammonium intermediate typically resulted in 15–25% recovery of the starting material.

When it comes to the synthesis of 3′-phosphoramidite monomer of 2-cyanoethyl-protected 5^{HOMe}dC, the above mentioned methods can not be utilized due to the presence of unprotected 3′-OH. In our experiments, the application of the biphasic 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl), Na_2CO_3, TBAB, CH_2Cl_2/H_2O/conc. NH_4OH method [20,21] afforded the desired product in only 53% (Fig. 1, Method A). In a recent report on the

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preparation of the oxidized 2′-*O*-methyl-^{5Me}C derivatives, TPSCl-based C^4-amination gave the desired products in only 42–67% yields [22].

Recently, Lakshman and coworkers reported a new approach to conduct C^4-amination on diTBS-protected thymidine by BOP/base/*N*-nucleophile system [23], which has been previously utilized for the preparation of N^6-modified adenosine [24,25] and C^6-modified guanosine derivatives [26]. Unfortunately, the effects of unprotected OH groups of ribose, oxidative thymine-modifications, and aminolysis with aqueous ammonia on this method were still unknown. In our initial trial, the employment of BOP/DBU/NH_3 in dioxane afforded the desired product. However, the 55% yield indicated that the application of this BOP-based amination method on oxidized ^{5Me}dU substrates was far from satisfactory (Fig. 1, Method B). Since it is well known that changes in the structures of phosphonium or benzotriazole moieties of onium salt-based coupling reagents may significantly affect their reactivity in peptide synthesis [27], we felt that it was quite important and necessary to reveal how the nature of onium salt-based coupling reagents, i.e. phosphonium vs aminium/uronium and OAt vs OBt, would affect the C^4-amination of pyrimidine nucleobases, especially more complicated 5-methyl-oxidized derivatives. Moreover, the formation of carcinogenic HMPA is another big concern with the current BOP-based method.

In this paper, a systematic and comparative study on the commonly used phosphonium and aminium/uronium coupling reagents lead us to the findings that different types of onium coupling reagents have distinctive reaction mechanisms and PyAOP is a much more potent reagent for the C^4-amination of thymine and 5-methyl-oxidized derivatives in both activation and aminolysis steps. Direct amination without the need of ribose protection, ultrafast activation, tolerance to aqueous *N*-nucleophiles, and excellent yields for various oxidized ^{5Me}dC derivatives are the advantages of the PyAOP-based method.

2. Results and discussion

2.1. The activation of thymine by different onium salt-based coupling reagents

In the preliminary experiment, we tested the activation capability of a series of commonly used onium salt-based coupling reagents. The results in Table 1 showed that in the presence of 2.0 equiv of PyAOP, PyBOP, AOP, and BOP and 2.0 equiv of DBU, the conversion of **1** to the corresponding OAt/OBt derivatives (**1a**/**1b**) was quite efficient (quant. to *ca.* 90%) in anhydrous THF ([**1**] = 0.3 M). But it can be seen that the reaction rate of PyAOP/PyBOP was significantly faster than that of AOP/BOP, indicating that tris(pyrrolidino)phosphonium-based reagents are more reactive than tris(dimethylamino)phosphonium-based ones. When the amounts of coupling reagents and DBU were reduced to 1.6 equiv, only PyAOP still could furnish quantitative activation of **1** in 1 min. A significant amount of **1** was left (*ca.* 20–30%) in the cases of the other three coupling reagents. It was obvious that the activation by

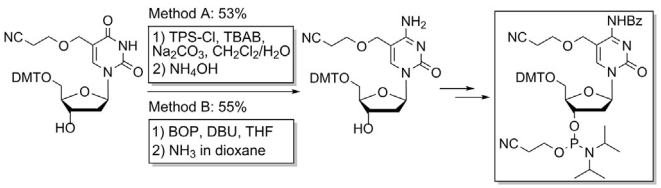
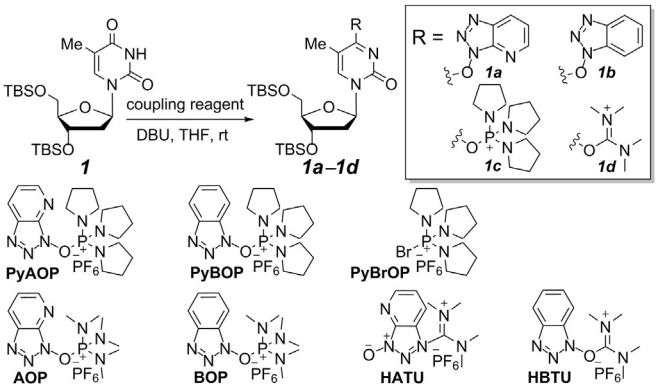


Fig. 1. C^4-Amination for the synthesis of a 3′-phosphoramidite monomer of 2-cyanoethyl-protected ^{5HOMe}dC.

Table 1
The activation of diTBS-dT (**1**) by onium salt-based coupling reagents.



Entry	Coupling reagent	Equiv	Reaction time (min)	Conversion (%)[a]
1.1	PyAOP	2.0	1	quant. (79)[b], **1a**
1.2	PyAOP	1.6	1	quant., **1a**
2.1	PyBOP	2.0	1	quant. (71)b, 1b
2.2	PyBOP	1.6	3	*ca.* 80, **1b**
3.1	AOP	2.0	10	*ca.* 90, **1a**
3.2	AOP	1.6	15	*ca.* 80, **1a**
4.1	BOP	2.0	10	*ca.* 90, **1b**
4.2	BOP	1.6	15	*ca.* 70, **1b**
5	PyBrOP	2.0	1	quant. (73)[b], **1c**
6	HATU	2.0	1	quant. (77)[b], **1d**
7	HBTU	2.0	1	quant., **1d**

[a] As assessed by TLC.
[b] Isolated yields.

OAt-based reagents was more efficient than that by OBt-based ones. Previously, Lakshman et al. failed to isolated **1b** from BOP-activated reaction due to decomposition [23]. In our work, we isolated both pure **1a** and **1b** in over 70% yields by flash column chromatography from PyAOP- and PyBOP-activated reactions. Interestingly, the reaction of PyBrOP with **1** quantitatively generated the phosphonium salt **1c**, which was isolated in 73% yield. To our surprise, the activation of **1** by both HATU and HBTU in the presence of DBU ended up with the formation of uronium salt **1d** instead of **1a**/**1b,** indicating that the stability of phosphonium and uronium intermediates are of huge difference. Flash column chromatography yielded pure **1d** in 77% yield.

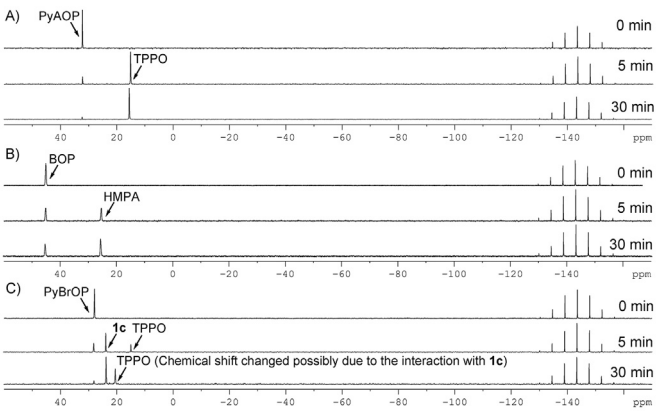


Fig. 2. ^{31}P NMR tracing of the activation of **1** by 2 equiv of PyAOP (A), BOP (B), and PyBrOP (C) and 2 equiv of DBU.

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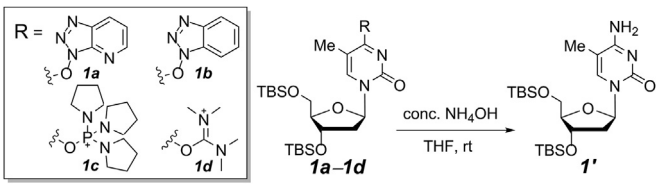
Table 2
The aminolysis of **1a**–**1d** by conc. NH_4OH.



Entry	Substrates	Equiv of conc. NH_4OH	Reaction time (h)	Isolated yields of 1′/1 (%)
1	**1a**	10	4	93/0
2	**1b**	10	9	88/3
3	**1c**	10	5	81/10
4	**1d**	20	12	0/21

^{31}P NMR tracing of PyAOP-, BOP-, and PyBrOP-based reactions (Fig. 2) confirmed that the activation by PyBrOP resulted in the formation of phosphonium salt **1c** (δ 23.6 ppm), whereas PyAOP and BOP directly lead to the formation of TPPO (δ 15.4 ppm) and HMPA (δ 25.4 ppm) [23,25]. In addition, PyAOP and PyBrOP almost disappeared at 30 min, whereas *ca.* 40% of BOP remained unreacted, suggesting that PyAOP and PyBrOP were more reactive and less stable than BOP in the presence of DBU.

2.2. The aminolysis of distinctive activated intermediates

The isolation of **1a**–**1d** provided a valuable chance to compare their reactivity in aminolysis step without the interference of activation step. As listed in Table 2, the reactions of OAt-based **1a** with aqueous ammonia (10 equiv) afforded diTBS-^{5Me}dC (**1′**) in 93% yield (4 h) without the recovery of **1**, suggesting that **1a** was quite resistant to hydrolysis ([**1a**] = 0.3 M). In contrast, the reaction of OBt-based **1b** was remarkably slower (9 h). Though yield of **1′** was also very good (88%), however, a trace amount of **1** was obtained due to hydrolysis of **1b.** As expected, phosphonium salt **1c** was also reactive to aqueous ammonia (5 h), however, **1′** was obtained in only 81% yield due to partial hydrolysis of **1c**. To our surprise, uronium salt **1d** was completely unreactive to NH_3. Treatment of **1d** with 20 equiv of conc. NH_4OH only recovered **1** (21%) via hydrolysis when the reaction was stopped at 12 h.

2.3. Comparison of PyAOP and BOP on the C^4-amination of simple dT and a more complicated oxidized ^{5Me}dU substrate

Further application of PyAOP for direct C^4-amination of OH-unprotected and base-modified pyrimidine nucleosides was conducted in a "one-pot, two-steps" manner along with BOP as a reference and anhydrous DMF as solvent. The results in Table 3 showed that PyAOP afforded ^{5Me}dC (**2′**) in excellent yield (92%). The activation of dT (**2**) in DMF by PyAOP (1.6 equiv) only needed 1 min ([**2**] = 0.3 M), and the subsequent aminolysis required less conc. NH_4OH (4 equiv) and reaction time (2 h) possibly due to the presence of DBU. The role of DBU in the activation step was also confirmed by replacing DBU with other bases. When DIPEA, TMEDA, DABCO, or Cs_2CO_3 was used as base, no reaction was observed, indicating that the deprotonation by strong base DBU was essential for the initiation of activation. In comparison, BOP also afforded **2′** in good yield (85%), but both steps were slower than those of PyAOP. However, in case of 2-cyanoethyl-protected 5′-*O*-DMT-^{5HOMe}dU (**3**), the activation by BOP was significantly much less efficient (*ca.* 60%, 10 min). After aminolysis, 2-cyanoethyl-protected 5′-DMT-^{5HOMe}dC (**3′**) was only obtained in 56% yield. ([**3**] was lowered from 0.3 M to 0.05 M to avoid deprotection of 2-cyanoethyl group.) In contrast, PyAOP well maintained its high potency in the activation of **3** (*ca.* 90%, 1 min), and **3′** was isolated in 84% yield. This result indicated that BOP is acceptable for C^4-amination of dT. However, in cases of structurally more complicated oxidized ^{5Me}dU substrates, the efficacy of BOP drops remarkably and more potent PyAOP is essential for efficient C^4-amination.

2.4. The PyAOP-based C^4-amination method for the preparation of a diversity of oxidized ^{5Me}dU substrates

To explore the generality of this PyAOP-based method, the C^4-amination was performed on a diversity of oxidized $^{5\text{-}Me}$dU derivatives including 2-cyanoethyl-protected $^{5\text{-}HOMe}$dU (**4**), $^{5\text{-}HOMe}$dU (**5**), diTBS-$^{5\text{-}AcOMe}$dU (**6**), $^{5\text{-}CHO}$dU (**7**), and $^{5\text{-}COOBn}$dU (**8**). As shown in Table 4, other than compounds **3** and **4** (0.05 M, 4 h), the reactions of all other substrates with conc. NH_4OH (4 equiv) were performed at 0.3 M and finished in only 2 h. For non-aqueous alkylamines (4 equiv), the aminolysis was much faster. While the reactions with benzylamine, morpholine, and piperidine completed in 5 min, those with diethylamine required 30 min to finish. PyAOP exhibited excellent reactivity to all these oxidized $^{5\text{-}Me}$dU substrates and yielded the corresponding products in 75–88% yields.

3. Conclusions

In summary, the low efficacy of existing C^4-amination methods including the newly reported BOP-based method for the preparation of oxidized $^{5\text{-}Me}$dC derivatives has urged us to systematically investigate how the nature of onium salt-based coupling reagents affects the C^4-amination of pyrimidine nucleobases. The comparative study on phosphonium and aminium/uronium coupling reagents revealed the advantages of tris(pyrrolidino)phosphonium over tris(dimethylamino) phosphonium and OAt over OBt in the activation step. In addition, different onium salt reagents resulted in the formation of distinctive activation intermediates. The aminolysis with conc. NH_4OH showed that OAt-based intermediate is not only resistant to hydrolysis but also more reactive than OBt-based one. Further experiments showed that the efficacy of BOP drops significantly in cases of structurally more complicated pyrimidine substrates, and more potent PyAOP is essential for efficient C^4-amination of a diversity of oxidized ^{5Me}dU substrates.

4. Experimental

4.1. General methods

General chemical reagents and solvents were obtained from commercial suppliers. Compounds **3**–**8** were synthesized according to reported methods [15,16,20]. All reactions were monitored by thin layer chromatography on plates coated with 0.25 mm silica gel 60 F_{254}. TLC plates were visualized by UV irradiation (254 nm) or by staining with 20% H_2SO_4 in EtOH. THF and DMF were distilled from CaH_2 prior to use. Flash column chromatography employed silica gel (particle size 32–63 μm). Melting points were determined with a Thomas-Hoover melting point apparatus and uncorrected. NMR spectra were obtained with a Bruker AV-400 instrument with chemical shifts reported in parts per million (ppm, δ) and referenced to $CDCl_3$, DMSO-d_6 or D_2O. IR spectra were recorded on a Bruker Vertex-70 spectrometer. Low- and high-resolution mass spectra were reported as *m/z* and obtained with a Bruker amaZon SL mass spectrometer and a Bruker Dalton micrOTOF-Q II

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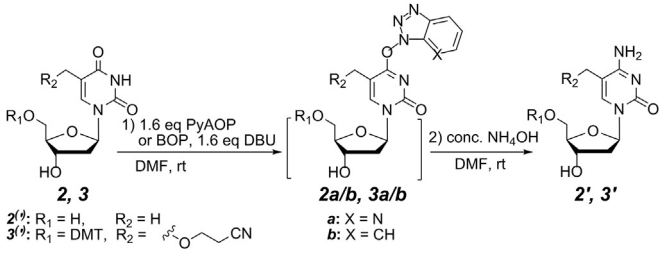
Table 3
The "one-pot, two-steps" C^4-amination of dT (**2**) and an oxidized 5′-*O*-DMT-^{5Me}dU derivative (**3**) with PyAOP or BOP.



No	Compd	Coupling reagent	Step 1 time (min)/ conv (%)[a]	Step 2 time (h)	Isolated yield (%)
1	**2**	PyAOP	1/quant.	2	92
2	**2**	BOP	5/90	3	85
3	**3**	PyAOP	1/*ca.* 90	4	84
4	**3**	BOP	10/*ca.* 60	5	56

[a] As assessed by TLC.

spectrometer, respectively.

4.2. General procedure for the synthesis of activated di-TBS-dT intermediates (1a–1d)

To a solution of diTBS-dT (**1**) in THF ([**1**] = 0.3 M) were added coupling reagent (PyAOP, PyBOP, PyBrOP, or HATU, 2 eq) and DBU (2 eq). The reaction was stirred at 20 °C for 1 min. Then the solution was concentrated *in vacuo*. Flash column chromatography on silica gel afforded **1a**–**1d** in pure form.

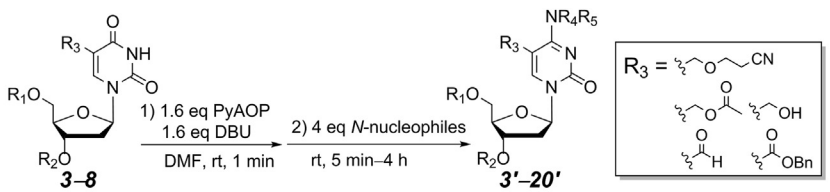
4.2.1. O^4-(7-Aza-1H-benzotriazol-1-yl)-3′,5′-di-O-(t-butyldimethylsilyl)thymidine (**1a**)

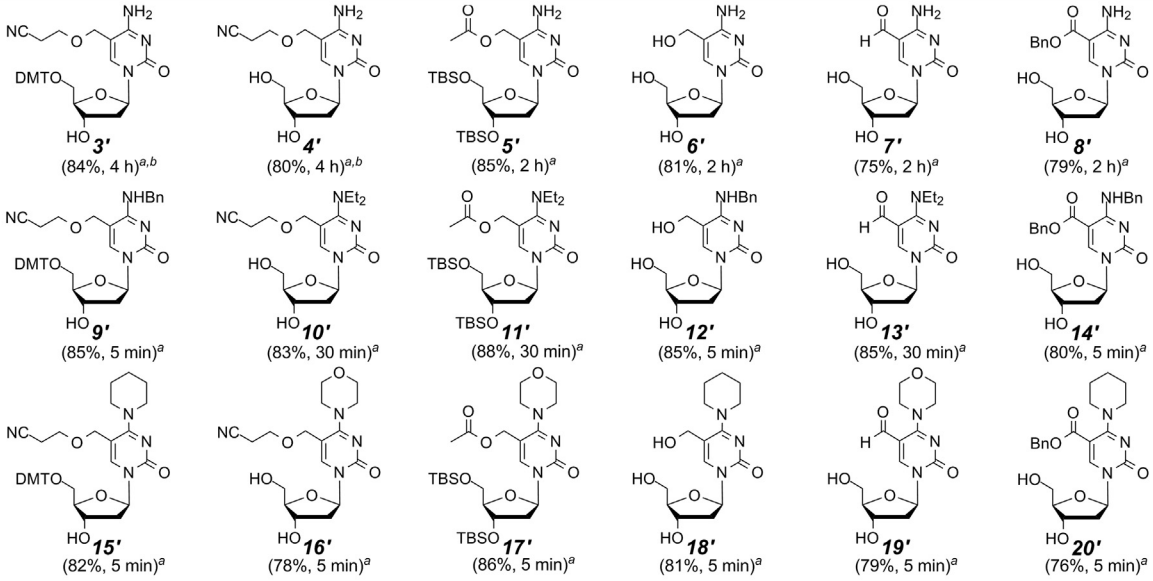
The reaction of **1** (94 mg, 0.2 mmol), PyAOP (208 mg, 0.4 mmol), and DBU (61 mg, 0.4 mmol) in THF (0.67 mL) afforded (PE/EA = 6:1) **1a** (93 mg, 79%) as a white solid; mp 184–185 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.70 (d, J = 4.4 Hz, 1H), 8.43 (d, J = 8.3 Hz, 1H), 8.16 (s, 1H), 7.47–7.41 (m, 1H), 6.15 (t, J = 6.1 Hz, 1H), 4.38–4.32 (m, 1H), 3.99–3.97 (m, 1H), 3.96–3.92 (m, 1H), 3.78 (d, J = 9.6 Hz, 1H), 2.50–2.42 (m, 1H), 2.30 (s, 3H), 2.04–1.96 (m, 1H), 0.95 (s, 9H), 0.87 (s, 9H), 0.14 (d, J = 6.8 Hz, 6H), 0.05 (s, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.0, 153.9, 151.8, 144.1, 140.8, 135.3, 129.8, 121.0, 100.8, 88.5, 87.4, 71.3, 62.6, 42.5, 26.1, 25.9, 18.6, 18.1, 12.0, −4.4, −4.7, −5.2 ppm; HRMS (ESI+): *m/z* calcd for $C_{27}H_{45}N_6O_5Si_2$ $[M+H]^+$ 589.2984; found 589.2980.

4.2.2. O^4-(1H-Benzotriazol-1-yl)-3′,5′-di-O-(t-butyldimethylsilyl) thymidine (**1b**)

The reaction of **1** (94 mg, 0.2 mmol), PyBOP (208 mg, 0.4 mmol), and DBU (61 mg, 0.4 mmol) in THF (0.67 mL) afforded (PE/EA = 6:1) **1b** (83 mg, 71%) as a white solid; mp 55–56 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (s, 1H), 8.06 (d, J = 8.3 Hz, 1H), 7.54–7.49 (m, 1H), 7.45–7.38 (m, 2H), 6.18 (t, J = 6.2 Hz, 1H), 4.39–4.34 (m, 1H), 4.01–3.98 (m, 1H), 3.98–3.93 (m, 1H), 3.82–3.76 (m, 1H), 2.54–2.46 (m, 1H), 2.28 (s, 3H), 2.05–1.97 (m, 1H), 0.95 (s, 9H), 0.87 (s, 9H), 0.14 (d, J = 6.3 Hz, 6H), 0.06 (s, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.0, 153.9, 144.0, 143.7, 129.0, 128.8, 124.9, 120.7, 108.9,

Table 4
The PyAOP-based C^4-amination method for oxidized ^{5Me}dC derivatives (**3′**–**20′**).





[a] (Isolated yield, reaction time of the 2nd step). [b] [**3**,**4**] = 0.05 M

100.7, 88.6, 87.5, 71.6, 62.7, 42.6, 26.1, 25.9, 18.6, 18.1, 12.0, −4.4, −4.7, −5.2 ppm; HRMS (ESI+): *m/z* calcd for $C_{28}H_{46}N_5O_5Si_2$ $[M+H]^+$ 588.3032; found 588.3026.

4.2.3. (1-(3′,5′-Di-O-(t-butyldimethylsilyl)-2′-deoxy-β-D-ribofuranosyl)-2-oxo-5-methyl-(1H)pyrimidin-4-yl-oxy) tripyrrolidinophosphonium hexafluorophosphate (**1c**)

The reaction of **1** (94 mg, 0.2 mmol), PyBrOP (186 mg, 0.4 mmol), and DBU (61 mg, 0.4 mmol) in THF (0.67 mL) afforded (PE/EA = 6:1) **1c** (125 mg, 73%) as a white solid; mp 67−68 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (s, 1H), 6.18 (t, *J* = 6.5 Hz, 1H), 4.38−4.33 (m, 1H), 4.02−3.98 (m, 1H), 3.88−3.82 (m, 1H), 3.77−3.72 (m, 1H), 3.38−3.30 (m, 12H), 2.54−2.46 (m, 1H), 2.04 (s, 3H), 2.02−1.97 (m, 1H), 1.97−1.90 (m, 12H), 0.86 (s, 9H), 0.85 (s, 9H), 0.07 (s, 6H), 0.04 (s, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.8, 164.7, 153.6, 145.2, 103.6, 103.5, 88.8, 87.7, 72.2, 62.9, 48.1, 42.3, 26.2, 26.1, 26.0, 25.8, 18.4, 18.0, 12.2, −4.6, −4.8, −5.4 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ 27.0 (s, 1P), −140.3 (m, 1P) ppm; HRMS (ESI+): *m/z* calcd for $C_{34}H_{65}N_5O_5PSi_2$ $[M]^+$ 710.4256; found 710.4250.

4.2.4. (1-(3′,5′-Di-O-(t-butyldimethylsilyl)-2′-deoxy-β-D-ribofuranosyl)-2-oxo-5-methyl-(1H)pyrimidin-4-yl-oxy)-N,N,N′,N′-tetramethyluronium hexafluorophosphate (**1d**)

The reaction of **1** (94 mg, 0.2 mmol), HATU (152 mg, 0.4 mmol), and DBU (61 mg, 0.4 mmol) in THF (0.67 mL) afforded (PE/EA = 6:1) **1d** (110 mg, 77%) as a white solid; mp 58−59 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 (s, 1H), 6.25 (t, *J* = 7.2 Hz, 1H), 4.41−4.37 (m, 1H), 3.96−3.93 (m, 1H), 3.86−3.81 (m, 1H), 3.77−3.71 (m, 1H), 3.39 (s, 6H), 3.10−2.99 (m, 6H), 2.34−2.26 (m, 1H), 2.19−2.10 (m, 1H), 1.94 (s, 3H), 0.91 (s, 9H), 0.87 (s, 9H) 0.10 (s, 6H), 0.07 (d, *J* = 3.3 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.4, 155.4, 147.4, 137.7, 110.4, 88.6, 86.3, 72.4, 63.2, 43.3, 40.9, 38.7, 26.1, 25.8, 18.5, 18.0, 12.7, −4.6, −4.8, −5.3 ppm; ^{31}P NMR (162 MHz, $CDCl_3$): δ −140.2 (m, 1P) ppm; HRMS (ESI+): *m/z* calcd for $C_{27}H_{53}N_4O_5Si_2$ $[M]^+$ 569.3549; found 569.3545.

4.3. General procedure for the synthesis of ^{5Me}dC and oxidized ^{5Me}dC derivatives (1′−20′)

To a solution of the nucleosides (**2**−**20**) in DMF ([substrate] = 0.3 M, except for **3** and **4,** [**3**,**4**] = 0.05 M) were added PyAOP (1.6 eq) and DBU (1.6 eq). The reaction was stirred at 20 °C for 1 min. To the reaction solution was added *N*-nucleophiles (4 eq) (or **1a** in THF, conc. NH_4OH (10 eq)). The reaction was stirred at 20 °C for 5 min−4 h and monitored by TLC. Upon completion, the solution was concentrated in *vacuo.* Flash column chromatography on silica gel afforded ^{5Me}dC and oxidized ^{5Me}dC derivatives (**1′**−**20′**) in pure form.

4.3.1. 3′,5′-Di-O-(t-butyldimethylsilyl)-5-methyl-2′-deoxycytidine (**1′**)

The reaction of **1a** (70 mg, 0.12 mmol) and conc. NH_4OH (45 μL, 1.2 mmol) in THF (0.4 mL) afforded (PE/EA = 2:1) **1′** (52 mg, 93%) as a white solid; mp 91−92 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49 (s, 1H), 6.28 (t, *J* = 6.6 Hz, 1H), 4.35−4.28 (m, 1H), 3.90−3.86 (m, 1H), 3.85−3.80 (m, 1H), 3.74−3.69 (m, 1H), 2.36−2.30 (m, 1H), 1.95−1.88 (m, 1H), 1.86 (s, 3H), 0.88 (s, 9H), 0.84 (s, 9H), 0.06 (d, *J* = 2.6 Hz, 6H), 0.02 (d, *J* = 1.6 Hz, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.8, 156.1, 137.9, 101.6, 87.6, 85.8, 71.9, 62.8, 42.1, 26.0, 25.8, 18.4, 18.0, 13.3, −4.5, −4.8, −5.3, −5.4 ppm; LRMS (ESI+): *m/z* calcd for $C_{22}H_{44}N_3O_4Si_2$ $[M+H]^+$ 470.3; found 470.3.

4.3.2. 5-Methyl-2′-deoxycytidine (**2′**)

The reaction of **2** (97 mg, 0.4 mmol), PyAOP (333 mg, 0.64 mmol), DBU (97 mg, 0.64 mmol), and conc. NH_4OH (62 μL, 1.6 mmol) in DMF (1.33 mL) afforded (CH_2Cl_2/MeOH = 8:1) **2′** (88 mg, 92%) as a white solid; mp 198−199 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.79 (s, 1H), 7.62 (br, 1H), 7.08 (br, 1H), 6.31 (t, *J* = 6.8 Hz, 1H), 5.43 (br, 2H), 4.36−4.33 (m, 1H), 3.91−3.87 (m, 1H), 3.76−3.64 (m, 2H), 2.24−2.17 (m, 1H), 2.14−2.05 (m, 1H), 1.98 (s, 3H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.2, 155.1, 138.5, 101.5, 87.3, 84.8, 70.5, 61.5, 40.4, 13.4 ppm; LRMS (ESI+): *m/z* calcd for $C_{10}H_{16}N_3O_4$ $[M+H]^+$ 242.1; found 242.1.

4.3.3. 5′-O-(4,4′-Dimethoxytrityl)-5-(2-cyanoethoxy)methyl-2′-deoxycytidine (**3′**)

The reaction of **3** (123 mg, 0.2 mmol), PyAOP (167 mg, 0.32 mmol), DBU (49 mg, 0.32 mmol), and conc. NH_4OH (31 μL, 0.8 mmol) in DMF (4 mL) afforded (CH_2Cl_2/MeOH = 20:1) **3′** (103 mg, 84%) as a white solid; mp 143−145 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (s, 1H), 7.39 (d, *J* = 7.4 Hz, 2H), 7.33−7.21 (m, 7H), 6.83 (d, *J* = 8.3 Hz, 4H), 6.51 (t, *J* = 6.5 Hz, 1H), 6.01 (br, 1H), 4.70 (br, 1H), 4.56−4.49 (m, 1H), 4.13 (d, *J* = 2.5 Hz, 1H), 3.78 (s, 6H), 3.73 (d, *J* = 12.4 Hz, 1H), 3.56−3.46 (m, 2H), 3.33−3.26 (m, 1H), 3.20−3.06 (m, 2H), 2.73−2.64 (m, 1H), 2.36−2.30 (m, 2H), 2.28−2.17 (m, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.2, 158.8, 156.2, 144.5, 140.7, 135.6, 135.5, 130.3, 130.2, 128.5, 128.1, 127.3, 117.6, 113.4, 102.2, 86.8, 86.6, 86.3, 72.1, 67.6, 64.0, 63.6, 55.4, 42.2, 18.5 ppm; LRMS (ESI+): *m/z* calcd for $C_{34}H_{37}N_4O_7$ $[M+H]^+$ 613.3; found 613.3.

4.3.4. 5-(2-Cyanoethoxy)methyl-2′-deoxycytidine (**4′**)

The reaction of **4** (93 mg, 0.3 mmol), PyAOP (250 mg, 0.48 mmol), DBU (73 mg, 0.48 mmol), and conc. NH_4OH (46 μL, 1.2 mmol) in DMF (6 mL) afforded (CH_2Cl_2/MeOH = 8:1) **4′** (74 mg, 80%) as a white solid; mp 176−177 °C. ^{1}H NMR (400 MHz, D_2O): δ 7.93 (s, 1H), 6.16 (t, *J* = 6.4 Hz, 1H), 4.43−4.40 (m, 1H), 4.39−4.36 (m, 2H), 4.04−3.96 (m, 1H), 3.85−3.77 (m, 1H), 3.76−3.72 (m, 1H), 3.71−3.66 (m, 2H), 2.75 (t, *J* = 5.9 Hz, 2H), 2.45−2.36 (m, 1H), 2.28−2.19 (m, 1H) ppm; ^{13}C NMR (100 MHz, D_2O): δ 164.6, 156.6, 142.1, 120.0, 103.5, 86.9, 86.4, 70.4, 65.6, 64.3, 61.2, 39.7, 18.3 ppm; IR (KBr): ν_{max} 3470, 2900, 2267, 1666, 1489, 1304, 1093 cm^{-1}; HRMS (ESI+): *m/z* calcd for $C_{13}H_{19}N_4O_5$ $[M+H]^+$ 311.1350; found 311.1347.

4.3.5. 3′,5′-Di-O-t-butyldimethylsilyl-5-acetoxymethyl-2′-deoxycytidine (**5′**)

The reaction of **5** (106 mg, 0.2 mmol), PyAOP (167 mg, 0.32 mmol), DBU (49 mg, 0.32 mmol), and conc. NH_4OH (31 μL, 0.8 mmol) in DMF (0.67 mL) afforded (PE/EA = 2:1) **5′** (89 mg, 85%) as a white solid; mp 87−88 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 (s, 1H), 6.22 (t, *J* = 6.0 Hz, 1H), 4.88−4.75 (m, 2H), 4.34−4.31 (m, 1H), 3.94−3.91 (m, 1H), 3.89−3.84 (m, 1H), 3.78−3.72 (m, 1H), 2.45−2.38 (m, 1H), 2.06 (s, 3H), 2.02−1.94 (m, 1H), 0.90 (s, 9H), 0.86 (s, 9H), 0.09 (s, 6H), 0.04 (s, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.6, 164.8, 156.1, 143.1, 101.4, 87.9, 86.3, 71.4, 62.7, 60.5, 42.2, 26.1, 25.9, 20.9, 18.5, 18.1 −4.5, −4.8, −5.3 ppm; IR (KBr): ν_{max} 3486, 2987, 1743, 1671, 1491, 1302, 1206, 845 cm^{-1}; HRMS (ESI+): *m/z* calcd for $C_{24}H_{46}N_3O_6Si_2$ $[M+H]^+$ 528.2920; found 528.2913.

4.3.6. 5-Hydroxymethyl-2′-deoxycytidine (**6′**)

The reaction of **6** (103 mg, 0.4 mmol), PyAOP (333 mg, 0.64 mmol), DBU (97 mg, 0.64 mmol), and conc. NH_4OH (62 μL, 1.6 mmol) in DMF (1.33 mL) afforded (CH_2Cl_2/MeOH = 5:1) **6′** (83 mg, 81%) as a white solid; mp 201−203 °C. ^{1}H NMR (400 MHz, D_2O): δ 7.91 (s, 1H), 6.22 (t, *J* = 6.4 Hz, 1H), 4.43 (s, 2H), 4.42−4.38 (m, 1H), 4.06−4.01 (m, 1H), 3.88−3.80 (m, 1H), 3.78−3.71 (m, 1H), 2.46−2.37 (m, 1H), 2.32−2.23 (m, 1H) ppm; ^{13}C NMR (100 MHz, D_2O): δ 164.2, 156.1, 140.8, 106.6, 86.8, 86.3, 70.4, 61.2, 57.7, 39.5 ppm; LRMS (ESI+): *m/z* calcd for $C_{10}H_{16}N_3O_5$ $[M+H]^+$ 258.1; found 258.1.

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*4.3.7. 5-Formyl-2′-deoxycytidine (**7′**)*

The reaction of **7** (102 mg, 0.4 mmol), PyAOP (333 mg, 0.64 mmol), DBU (97 mg, 0.64 mmol), and conc. NH_4OH (62 μL, 1.6 mmol) in DMF (1.33 mL) afforded (CH_2Cl_2/MeOH = 7:1) **7′** (76 mg, 75%) as a white solid; mp 193–195 °C. ^{1}H NMR (400 MHz, D_2O): δ 9.49 (s, 1H), 8.84 (s, 1H), 6.15 (t, *J* = 6.0 Hz, 1H), 4.45–4.38 (m, 1H), 4.14–4.10 (m, 1H), 3.93–3.87 (m, 1H), 3.80–3.73 (m, 1H), 2.60–2.51 (m, 1H), 2.39–2.31 (m, 1H) ppm; ^{13}C NMR (100 MHz, D_2O): δ 190.2, 161.7, 154.9, 153.8, 105.5, 87.7, 87.4, 69.9, 60.8, 57.7, 40.2 ppm; LRMS (ESI+): *m/z* calcd for $C_{10}H_{14}N_3O_5$ $[M+H]^+$ 256.1; found 256.1.

*4.3.8. 5-Benzyloxycarbonyl-2′-deoxycytidine (**8′**)*

The reaction of **8** (109 mg, 0.3 mmol), PyAOP (250 mg, 0.48 mmol), DBU (73 mg, 0.48 mmol), and conc. NH_4OH (46 μL, 1.2 mmol) in DMF (1 mL) afforded (CH_2Cl_2/MeOH = 10:1) **8′** (85 mg, 79%) as a white solid; mp 165–167 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.95 (s, 1H), 7.98–7.96 (m, 1H), 7.64–7.62 (m, 1H), 7.48–7.30 (m, 5H), 6.06 (t, *J* = 6.2 Hz, 1H), 5.26 (s, 2H), 5.26 (s, 1H), 5.08–5.04 (m, 1H), 4.26–4.19 (m, 1H), 3.92–3.86 (m, 1H), 3.64–3.53 (m, 2H), 2.33–2.25 (m, 1H), 2.08–2.01 (m, 1H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.5, 163.0, 153.2, 148.6, 136.2, 128.5, 128.0, 127.7, 94.4, 88.1, 86.7, 70.2, 65.7, 61.0, 41.3 ppm; IR (KBr): ν_{max} 3486, 2968, 1705, 1642, 1512, 1324, 1204 cm^{-1}; HRMS (ESI+): *m/z* calcd for $C_{17}H_{20}N_3O_6$ $[M+H]^+$ 362.1347; found 362.1347.

4.3.9. N^4-Benzyl-5′-O-(4,4′-dimethoxytrityl)-5-(2-cyanoethoxy) methyl-2′-deoxycytidine (9′)

The reaction of **3** (123 mg, 0.2 mmol), PyAOP (167 mg, 0.32 mmol), DBU (49 mg, 0.32 mmol), and benzylamine (86 mg, 0.8 mmol) in DMF (0.67 mL) afforded (CH_2Cl_2/MeOH = 25:1) **9′** (119 mg, 85%) as a white solid; mp 89–91 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (s, 1H), 7.40–7.36 (m, 2H), 7.34–7.30 (m, 4H), 7.29–7.24 (m, 8H), 6.83 (d, *J* = 8.1 Hz, 4H), 6.48 (t, *J* = 6.4 Hz, 1H), 6.15 (t, *J* = 5.5 Hz, 1H), 4.70 (d, *J* = 5.6 Hz, 2H), 4.62 (br, 1H), 4.16 (s, 1H), 4.14–4.12 (m, 1H), 3.78 (s, 6H), 3.76–3.71 (m, 1H), 3.59–3.53 (m, 1H), 3.44 (d, *J* = 12.8 Hz, 1H), 3.35–3.30 (m, 1H), 3.15–3.13 (m, 2H), 2.68–2.60 (m, 1H), 2.32–2.26 (m, 1H), 2.25–2.21 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.8, 158.9, 156.2, 144.4, 139.9, 138.2, 135.7, 135.6, 130.4, 130.3, 128.8, 128.5, 128.1, 127.6, 127.3, 117.4, 113.4, 101.9, 86.8, 88.2, 86.1, 71.8, 67.8, 63.7, 63.4, 55.4, 46.4, 42.3, 18.5 ppm; IR (KBr): ν_{max} 3465, 2968, 2242, 1680, 1564, 1250 cm^{-1}; HRMS (ESI+): *m/z* calcd for $C_{41}H_{43}N_4O_7$ $[M+H]^+$ 703.3126; found 703.3125.

*4.3.10. N^4,N^4-Diethyl-5-(2-cyanoethoxy)methyl-2′-deoxycytidine (**10′**)*

The reaction of **4** (93 mg, 0.3 mmol), PyAOP (250 mg, 0.48 mmol), DBU (73 mg, 0.48 mmol), and diethylamine (88 mg, 1.2 mmol) in DMF (1 mL) afforded (CH_2Cl_2/MeOH = 8:1) **10′** (91 mg, 83%) as a white solid; mp 188–189 °C. ^{1}H NMR (400 MHz, D_2O): δ 7.91 (s, 1H), 6.19 (t, *J* = 6.7 Hz, 1H), 4.44–4.40 (m, 1H), 4.38 (s, 2H), 4.04–4.01 (m, 1H), 3.88–3.78 (m, 2H), 3.77–3.73 (m, 2H), 3.65–3.58 (m, 4H), 2.77 (t, *J* = 5.8 Hz, 2H), 2.46–2.37 (m, 1H), 2.29–2.23 (m, 1H), 1.22–1.16 (m, 6H) ppm; ^{13}C NMR (100 MHz, D_2O): δ 162.0, 156.2, 145.0, 119.8, 104.1, 86.7, 86.0, 70.4, 69.4, 64.1, 61.2, 44.2, 39.6, 18.3, 12.9 ppm; IR (KBr): ν_{max} 3478, 2971, 2232, 1650, 1519, 1293, 1095 cm^{-1}; HRMS (ESI+): *m/z* calcd for $C_{17}H_{27}N_4O_5$ $[M+H]^+$ 367.1976; found 367.1972.

*4.3.11. N^4,N^4-Diethyl-3′,5′-di-O-t-butyldimethylsilyl-5-acetoxy-methyl-2′-deoxycytidine (**11′**)*

The reaction of **5** (106 mg, 0.2 mmol), PyAOP (167 mg, 0.32 mmol), DBU (49 mg, 0.32 mmol), and diethylamine (58 mg, 0.8 mmol) in DMF (0.67 mL) afforded (PE/EA = 4:1) **11′** (103 mg, 88%) as a white solid; mp 81–82 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (s, 1H), 6.29 (t, *J* = 6.5 Hz, 1H), 4.79 (s, 2H), 4.36–4.33 (m, 1H), 3.94–3.88 (m, 1H), 3.84–3.72 (m, 2H), 3.65–3.48 (m, 4H), 2.46–2.38 (m, 1H), 2.06 (s, 3H), 2.02–1.94 (m, 1H), 1.21 (t, *J* = 7.0 Hz, 6H), 0.88 (s, 9H), 0.87 (s, 9H), 0.07 (s, 6H), 0.05 (s, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 162.6, 154.6, 146.1, 100.5, 87.9, 86.2, 72.1, 63.8, 63.1, 43.8, 42.1, 26.0, 25.9, 21.2, 18.5, 18.1, 13.8, −4.5, −4.8, −5.3 ppm; IR (KBr): ν_{max} 2954, 2929, 2857, 1743, 1665, 1513, 1472, 1255, 837 cm^{-1}; HRMS (ESI+): *m/z* calcd for $C_{28}H_{54}N_3O_6Si_2$ $[M+H]^+$ 584.3546; found 584.3545.

*4.3.12. N^4-Benzyl-5-hydroxymethyl-2′-deoxycytidine (**12′**)*

The reaction of **6** (103 mg, 0.4 mmol), PyAOP (333 mg, 0.64 mmol), DBU (97 mg, 0.64 mmol), and benzylamine (171 mg, 1.6 mmol) in DMF (1.33 mL) afforded (CH_2Cl_2/MeOH = 5:1) **12′** (118 mg, 85%) as a white solid; mp 183–184 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.76 (s, 1H), 7.55 (t, *J* = 5.9 Hz, 1H), 7.34–7.28 (m, 4H), 7.25–7.21 (m, 1H), 6.17 (t, *J* = 6.5 Hz, 1H), 5.20 (d, *J* = 4.2 Hz, 1H), 5.10 (t, *J* = 5.3 Hz, 1H), 4.99 (t, *J* = 5.2 Hz, 1H), 4.57 (d, *J* = 6.0 Hz, 2H), 4.27 (d, *J* = 5.2 Hz, 2H), 4.21–4.18 (m, 1H), 3.79–3.76 (m, 1H), 3.61–3.52 (m, 2H), 2.12–2.05 (m, 1H), 1.99–1.91 (m, 1H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.2, 154.9, 139.4, 138.4, 128.3, 127.1, 126.7, 106.3, 87.2, 84.8, 70.5, 61.5, 57.6, 43.2, 40.3 ppm; IR (KBr): ν_{max} 3443, 2883, 1565, 1508, 1056 cm^{-1}; HRMS (ESI+): *m/z* calcd for $C_{17}H_{22}N_3O_5$ $[M+H]^+$ 348.1554; found 348.1549.

*4.3.13. N^4,N^4-Diethyl-5-formyl-2′-deoxycytidine (**13′**)*

The reaction of **7** (102 mg, 0.4 mmol), PyAOP (333 mg, 0.64 mmol), DBU (97 mg, 0.64 mmol), and diethylamine (117 mg, 1.6 mmol) in DMF (1.33 mL) afforded (CH_2Cl_2/MeOH = 8:1) **13′** (106 mg, 85%) as a white solid; mp 156–157 °C. ^{1}H NMR (400 MHz, D_2O): δ 9.44 (s, 1H), 8.70 (s, 1H), 6.14 (t, *J* = 6.1 Hz, 1H), 4.43–4.38 (m, 1H), 4.11–4.06 (m, 1H), 3.89–3.83 (m, 1H), 3.78–3.72 (m, 1H), 3.57–3.49 (m, 4H), 2.56–2.47 (m, 1H), 2.36–2.27 (m, 1H), 1.20 (t, *J* = 7.0 Hz, 6H) ppm; ^{13}C NMR (100 MHz, D_2O): δ 187.9, 159.8, 154.4, 153.1, 108.8, 87.2, 70.1, 60.9, 45.0, 40.2, 12.0 ppm; IR (KBr): ν_{max} 3465, 2975, 1564, 1508, 1250, 1088 cm^{-1}; HRMS (ESI+): *m/z* calcd for $C_{14}H_{22}N_3O_5$ $[M+H]^+$ 312.1554; found 312.1551.

*4.3.14. N^4-Benzyl-5-benzyloxycarbonyl-2′-deoxycytidine (**14′**)*

The reaction of **8** (109 mg, 0.3 mmol), PyAOP (250 mg, 0.48 mmol), DBU (73 mg, 0.48 mmol), and benzylamine (128 mg, 1.2 mmol) in DMF (1 mL) afforded (CH_2Cl_2/MeOH = 8:1) **14′** (108 mg, 80%) as a white solid; mp 147–148 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.97 (s, 1H), 8.68 (t, *J* = 5.8 Hz, 1H), 7.47–7.22 (m, 10H), 6.06 (t, *J* = 6.1 Hz, 1H), 5.27 (s, 2H), 5.26 (s, 1H), 5.07 (t, *J* = 4.8 Hz, 1H), 4.62 (d, *J* = 5.9 Hz, 2H), 4.25–4.20 (m, 1H), 3.91–3.86 (m, 1H), 3.64–3.54 (m, 2H), 2.33–2.25 (m, 1H), 2.09–2.01 (m, 1H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.7, 160.7, 153.1, 148.2, 138.6, 136.1, 128.5, 128.4, 128.0, 127.7, 127.4, 127.0, 94.6, 88.1, 86.7, 70.2, 65.8, 61.0, 43.4, 41.2 ppm; IR (KBr): ν_{max} 3485, 2900, 1650, 1572, 1495, 1065 cm^{-1}; HRMS (ESI+): *m/z* calcd for $C_{24}H_{26}N_3O_6$ $[M+H]^+$ 452.1816; found 452.1816.

*4.3.15. 1-(5′-O-(4,4′-Dimethoxytrityl))-2′-deoxy-β-D-ribofuranosyl)-5-(2-cyanoethoxy)methyl-4-(piperidin-1-yl)-2(1H)-pyrimidinone (**15′**)*

The reaction of **3** (123 mg, 0.2 mmol), PyAOP (167 mg, 0.32 mmol), DBU (49 mg, 0.32 mmol), and piperidine (68 mg, 0.8 mmol) in DMF (0.67 mL) afforded (CH_2Cl_2/MeOH = 20:1) **15′** (112 mg, 82%) as a white solid; mp 95–96 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (s, 1H), 7.46–7.39 (m, 2H), 7.33–7.21 (m, 7H), 6.86–6.81 (m, 4H), 6.45 (t, *J* = 6.2 Hz, 1H), 4.60–4.55 (m, 1H), 4.52 (br, 1H), 4.17–4.12 (m, 1H), 3.82 (s, 1H), 3.79 (s, 6H), 3.71–3.66 (m, 4H), 3.55 (d, *J* = 11.6 Hz, 2H), 3.27–3.22 (m, 1H), 2.95 (t, *J* = 6.2 Hz,

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2H), 2.70–2.61 (m, 1H), 2.27–2.19 (m, 1H), 2.13 (t, $J = 6.1$ Hz, 2H), 1.67–1.58 (m, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.1, 158.7, 155.3, 144.9, 144.7, 135.8, 135.6, 130.3, 130.2, 128.3, 128.0, 127.1, 117.6, 113.3, 103.5, 86.5, 86.3, 86.2, 71.9, 69.0, 64.1, 63.5, 55.3, 48.4, 42.3, 26.4, 24.6, 18.3 ppm; IR (KBr): ν_{max} 3485, 2900, 2264, 1651, 1507, 1250, 1065 cm^{-1}; HRMS (ESI+): *m/z* calcd for $C_{39}H_{45}N_4O_7$ $[M+H]^+$ 681.3283; found 681.3279.

*4.3.16. 1-(2′-Deoxy-β-D-ribofuranosyl)-5-(2-cyanoethoxy)methyl-4-(morpholin-4-yl)-2(1H)-pyrimidinone (**16′**)*

The reaction of **4** (93 mg, 0.3 mmol), PyAOP (250 mg, 0.48 mmol), DBU (73 mg, 0.48 mmol), and morpholine (104 mg, 1.2 mmol) in DMF (1 mL) afforded (CH_2Cl_2/MeOH = 12:1) **16′** (89 mg, 78%) as a white solid; mp 191–193 °C. ^{1}H NMR (400 MHz, D_2O): δ 8.02 (s, 1H), 6.20 (t, $J = 6.3$ Hz, 1H), 4.46–4.40 (m, 3H), 4.08–4.03 (m, 1H), 3.86–3.79 (m, 8H), 3.78–3.73 (m, 4H), 2.79 (t, $J = 5.7$ Hz, 2H), 2.49–2.42 (m, 1H), 2.34–2.26 (m, 1H) ppm; ^{13}C NMR (100 MHz, D_2O): δ 163.9, 156.4, 145.6, 119.9, 104.6, 86.9, 86.3, 70.3, 68.4, 66.6, 64.3, 61.1, 47.3, 39.7, 18.3 ppm; IR (KBr): ν_{max} 3474, 2908, 2255, 1649, 1506, 1241 cm^{-1}; HRMS (ESI+): *m/z* calcd for $C_{17}H_{25}N_4O_6$ $[M+H]^+$ 381.1769; found 381.1769.

*4.3.17. 1-(3′,5′-Di-O-t-butyldimethylsilyl-2′-deoxy-β-D-ribofuranosyl)-5-acetoxymethyl-4-(morpholin-4-yl)-2(1H)-pyrimidinone (**17′**)*

The reaction of **5** (106 mg, 0.2 mmol), PyAOP (167 mg, 0.32 mmol), DBU (49 mg, 0.32 mmol), and morpholine (70 mg, 0.8 mmol) in DMF (0.67 mL) afforded (PE/EA = 4:1) **17′** (102 mg, 86%) as a white solid; mp 78–79 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (s, 1H), 6.25 (t, $J = 6.4$ Hz, 1H), 4.86–4.74 (m, 2H), 4.37–4.31 (m, 1H), 3.92–3.98 (m, 1H), 3.81–3.76 (m, 2H), 3.75–3.68 (m, 8H), 2.50–2.43 (m, 1H), 2.06 (s, 3H), 2.01–1.93 (m, 1H), 0.88 (s, 9H), 0.87 (s, 9H), 0.07 (s, 6H), 0.05 (s, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 164.8, 154.7, 146.5, 101.7, 88.2, 86.7, 72.1, 66.9, 63.1, 62.4, 48.0, 42.1, 26.0, 25.9, 21.1, 18.5, 18.1, −4.5, −4.8, −5.3 ppm; IR (KBr): ν_{max} 2954, 2929, 1651, 1501, 1225, 1028, 838 cm^{-1}; HRMS (ESI+): *m/z* calcd for $C_{17}H_{25}N_4O_6$ $[M+H]^+$ 598.3338; found 598.3330.

*4.3.18. 1-(2′-Deoxy-β-D-ribofuranosyl)-5-hydroxymethyl-4-(piperidin-1-yl)-2(1H)-pyrimidinone (**18′**)*

The reaction of **6** (103 mg, 0.4 mmol), PyAOP (333 mg, 0.64 mmol), DBU (97 mg, 0.64 mmol), and piperidine (136 mg, 1.6 mmol) in DMF (1.33 mL) afforded (CH_2Cl_2/MeOH = 4:1) **18′** (105 mg, 81%) as a white solid; mp 189–190 °C. ^{1}H NMR (400 MHz, D_2O): δ 7.93 (s, 1H), 6.20 (t, $J = 6.4$ Hz, 1H), 4.45 (s, 2H), 4.44–4.39 (m, 1H), 4.05–4.01 (m, 1H), 3.86–3.80 (m, 1H), 3.77–3.73 (m, 1H), 3.72–3.67 (m, 4H), 2.45–2.37 (m, 1H), 2.32–2.23 (m, 1H), 1.65 (s, 6H) ppm; ^{13}C NMR (100 MHz, D_2O): δ 161.7, 154.8, 143.9, 108.2, 86.9, 86.1, 70.5, 61.2, 59.7, 49.1, 39.5, 25.9, 23.6 ppm; IR (KBr): ν_{max} 3462, 2937, 1643, 1504, 1460, 1072 cm^{-1}; HRMS (ESI+): *m/z* calcd for $C_{15}H_{24}N_3O_5$ $[M+H]^+$ 326.1710; found 326.1708.

*4.3.19. 1-(2′-Deoxy-β-D-ribofuranosyl)-5-formyl-4-(morpholin-4-yl)-2(1H)-pyrimidinone (**19′**)*

The reaction of **7** (102 mg, 0.4 mmol), PyAOP (333 mg, 0.64 mmol), DBU (97 mg, 0.64 mmol), and morpholine (139 mg, 1.6 mmol) in DMF (1.33 mL) afforded (CH_2Cl_2/MeOH = 8:1) **19′** (103 mg, 79%) as a white solid; mp 188–189 °C. ^{1}H NMR (400 MHz, D_2O): δ 9.38 (s, 1H), 8.84 (s, 1H), 6.16 (t, $J = 6.0$ Hz, 1H), 4.46–4.41 (m, 1H), 4.15–4.10 (m, 1H), 3.92–3.86 (m, 1H), 3.83–3.79 (m, 4H), 3.78–3.74 (m, 1H), 3.71–3.65 (m, 4H), 2.60–2.53 (m, 1H), 2.40–2.32 (m, 1H) ppm; ^{13}C NMR (100 MHz, D_2O): δ 187.5, 160.6, 155.8, 154.5, 108.4, 87.5, 87.4, 70.0, 66.4, 60.8, 48.8, 40.2 ppm; IR (KBr): ν_{max} 3482, 2986, 1685, 1518, 1205, 1064 cm^{-1}; HRMS (ESI+): *m/z* calcd for $C_{14}H_{20}N_3O_6$ $[M+H]^+$ 326.1347; found 326.1341.

*4.3.20. 1-(2′-Deoxy-β-D-ribofuranosyl)-5-benzyloxycarbonyl-4-(piperidin-1-yl)-2(1H)-pyrimidinone (**20′**)*

The reaction of **8** (109 mg, 0.3 mmol), PyAOP (250 mg, 0.48 mmol), DBU (73 mg, 0.48 mmol), and piperidine (102 mg, 1.2 mmol) in DMF (1.0 mL) afforded (CH_2Cl_2/MeOH = 10:1) **20′** (98 mg, 76%) as a white solid; mp 153–154 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.66 (s, 1H), 7.47–7.31 (m, 5H), 6.07 (t, $J = 6.3$ Hz, 1H), 5.28–5.24 (m, 1H), 5.23 (s, 2H), 5.08–5.04 (m, 1H), 4.25–4.20 (m, 1H), 3.87–3.83 (m, 1H), 3.62–3.52 (m, 2H), 3.41–3.36 (m, 4H), 2.28–2.21 (m, 1H), 2.08–1.99 (m, 1H), 1.56 (s, 2H), 1.49 (s, 4H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.1, 161.0, 152.8, 148.1, 136.0, 128.5, 128.2, 98.7, 87.9, 86.1, 70.4, 66.2, 61.2, 48.2, 41.0, 25.4, 23.7 ppm; IR (KBr): ν_{max} 3473, 2942, 1648, 1517, 1256, 1065 cm^{-1}; HRMS (ESI+): *m/z* calcd for $C_{22}H_{28}N_3O_6$ $[M+H]^+$ 430.1973; found 430.1965.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tet.2018.10.046.

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