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Synthesis of nucleoside aminooxy acids

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ABSTRACT

First nucleoside aminooxy acids were synthesized from furanoid sugar phthalimidooxy acids by N-glycosylation with uracil, thymine, *N*-benzoylcytosine, 6-*N*-benzoyladenine and 2-*N*-acetyl-6-*O*-diphenylcarbamoylguanine. Boc or Fmoc protected uridine aminooxy acid derivatives have also been prepared. As oxyamine protecting group, the phthalimido group was shown to be instable in MeOH, leading to the imide ring-opening product in a reversible way. This reaction was accelerated under acid or basic conditions. A uridine dimer linked by *N*-oxy amide has also been prepared by coupling of uridine aminooxy ester with uridine phthalimidooxy acid. These nucleoside aminooxy acids might constitute useful building blocks for the development of novel RNA mimics and conjugates with other biomolecules or reporter compounds.

1. Introduction

Much recent efforts have been devoted to the development of synthetic oligonucleotides for various therapeutic and diagnostic applications because of their capability to cause selective inhibition of gene expression by binding to the target DNA/RNA sequences through mechanisms, such as antigen, antisense and RNA interference.[1] Nonionic analogues of nucleic acids are promising for the treatment of viral diseases and cancer since nonionic phosphate mimics could increase cellular permeability and resistance to extra- and intracellular nucleases. A number of phosphodiester replacements have been reported, including amide,[2] thioacetamide,[3] triazole,[4] sulfide,[5] formacetal,[6] thioformacetal,[6b,7] dimethylenesulfone,[8] methylene(methylimino),[9] as well as silyl[10] analogues.

Synthesis of modified RNA oligomers has attracted renewed interest since the discovery of small interfering RNA (siRNA) for gene regulation.[1c,e,11] We are interested in the development of novel nucleoside building blocks, especially ribonucleoside aminooxy acid, which could be further used for the generation of *N*-oxy amide-linked oligoribonucleosides as novel oligonucleotide mimics (Fig. 1). Recent studies on aminooxy acids showed that aminooxypeptides can easily form intramolecular hydrogen bonds with turns and helices structures, and a new family of foldamers has been developed from α-, β- and γ-aminooxy acids.[12] Furthermore, *N*-oxy amide linkage is stable to chemical and enzymatic hydrolysis. Very recently, we[13] and others[14] have developed glycoaminooxy acids as a new class of sugar building blocks, with interesting secondary structure for their oligomers.[14] Aminooxy functionalized nucleoside[9,15] and oligonucleotides[16] have been reported for the development of methylene(methylimino)-linked oligonucleotide mimics, for prodrug design or for immobilization in the fabrication of microarrays. An *N*-oxy amide-linked thymidine dimer has been incorporated into DNA oligomer, which annealed to complementary DNA with nearly the same affinity as the natural sequence.[17] To the best of our knowledge, *N*-oxy amide-linked ribonucleosides have never been reported before. Like amide

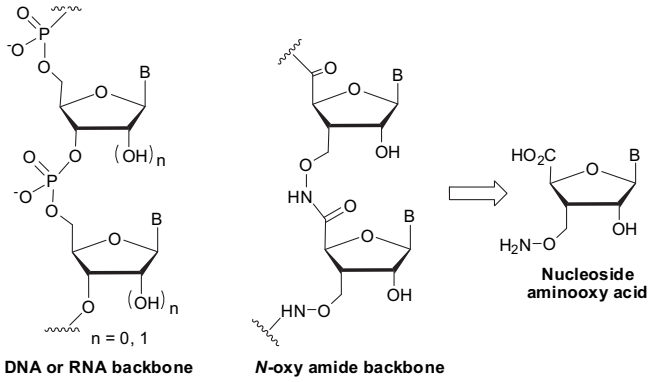

Fig. 1. Structure of natural and modified oligonucleotide backbones and nucleoside aminooxy acid.

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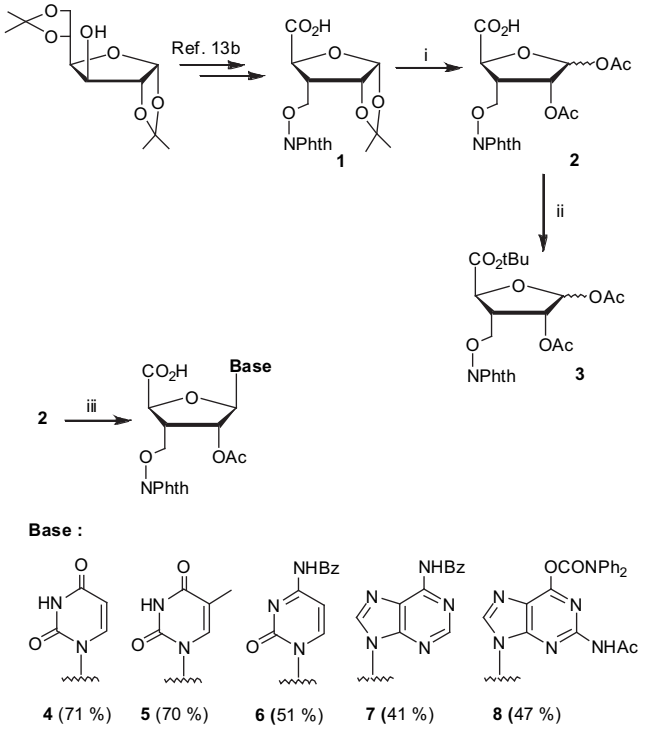
oligonucleosides, *N*-oxy amide-linked ones should be resistant to nucleases degradation. The stability towards peptidases, the easy formation of hydrogen bond and the possibility of further functionalization of *N*-oxy amide bond[18] are among the particularity of *N*-oxy amide derivatives compared to their amide counterparts.

Like amide-linked oligonucleotides, oligo *N*-oxy amide analogues of nucleic acids could be prepared with straightforward peptide coupling chemistry.[12] The synthetic challenge for the development of *N*-oxy amide-linked RNA is the synthesis of the nucleoside aminooxy acid building blocks (Fig. 1). We reported herein the synthesis of first nucleoside aminooxy acids. As a proof of principle, a dimer resulting from the monomers is described. Apart from oligomerization into oligonucleosides, several applications of nucleoside aminoxy acids described herein can be envisaged. Thanks to the presence of both aminooxyl and carboxyl functions, these nucleosides can be readily used for oligonucleotide conjugation with peptides or carbohydrates,[19] which might find applications for viral infections and/or cancer treatment.[20] Nucleoside aminooxy acids might also be suitable for conjugations with reporter compounds and markers.

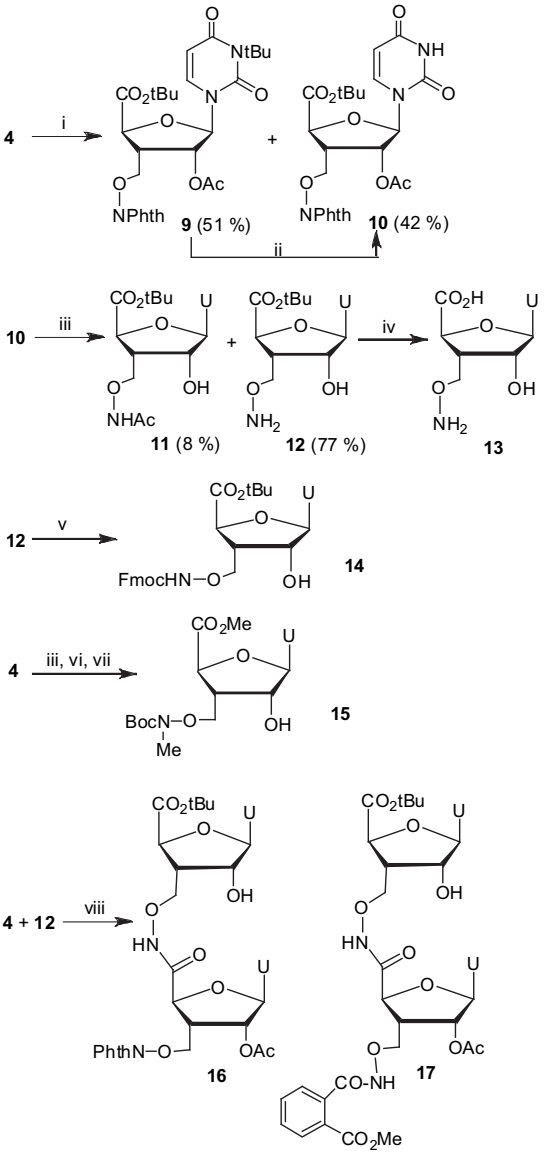
2. Results and discussion

Nucleoside aminooxy acids might be prepared either from natural nucleosides, by introducing aminooxy acid function on the sugar ring, or from furanoid sugar aminooxy acids by N-glycosylation with nucleic bases. After unsuccessful ring opening of the 2,3′-anhydro uridine derivative with *N*-protected hydroxylamine, we then decided to start the synthesis from our previously prepared furanoid sugar aminooxy acid **1** (Scheme 1).[13b] Removal of the isopropylidene group under acidic condition followed by acetylation with Ac_2O led to **2**, which can be readily transformed into ester **3**. N-Glycosylation of **2** with uracil, thymine, *N*-benzoylcytosine, 6-*N*-benzoyladenine and 2-*N*-acetyl-6-*O*-diphenylcarbamoylguanine[21] in the presence of *N*,*O*-bis(trimethylsilyl)acetamide (BSA) and TMSOTf under reflux led successfully to the corresponding nucleoside aminooxy acids **4**–**8** in 41–71% yields. 1,2-Dichloroethane was used as solvent for the synthesis of **10** because of insolubility of 2-*N*-acetyl-6-*O*-diphenylcarbamoylguanine in acetonitrile. The relative low yields for the nucleoside formation is due to the loss of product during purification, despite the fact that all the glycosylations were clean on TLC.

In order to make nucleoside aminooxy acids suitable for further transformations, we then tried to introduce different protecting groups on the uridine derivative **4** (Scheme 2). Esterification of **4** with *tert*-butoxytrichloroacetimidate led not only to the desired ester **10** but also the *N*-*tert*-butyl compound **9**, which can be converted into **10** by treatment with AcOH in CH_2Cl_2. Hydrazinolysis of **10** led to the oxyamine **12** and a small quantity of the transacetylation product **11**. Acidic deprotection of **12** led to the fully deprotected aminooxy acid **13**, which was somewhat instable[9e] and difficult to be purified. Nevertheless, its structure has been



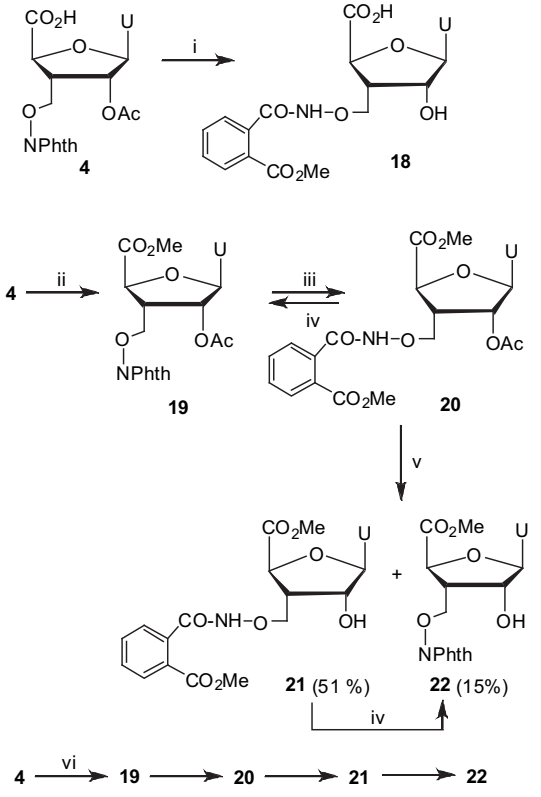
Scheme 1. Reagents and conditions: (i) H_2SO_4, Ac_2O, AcOH, 77%; (ii) *t*-BuOH, DCC, DMAP, CH_2Cl_2, 60%; (iii) base: uracil for **4**, thymine for **5**, *N*-benzoylcytosine for **6**, 6-*N*-benzoyladenine for **7**, 2-*N*-acetyl-6-*O*-diphenylcarbamoylguanine for **8**; BSA, TMSOTf, MeCN (for **4** to **7**) or $ClCH_2CH_2Cl$ (for **8**).



Scheme 2. Reagents and conditions: (i) *t*-BuOC(=NH)CCl_3 (2.6 equiv), cyclohexane, CH_2Cl_2; (ii) 10% AcOH, CH_2Cl_2, 85%; (iii) NH_2NH_2, MeOH; (iv) TFA, CH_2Cl_2, 100%; (v) FmocOSu, $NaHCO_3$, 1,4-dioxane, H_2O, 73%; (vi) Boc_2O, $NaHCO_3$, 1,4-dioxane, H_2O; (vii) MeI, $NaHCO_3$, 25% for three steps; (viii) EDC, HOBt, DMF, 50% from **10**.

confirmed by ^{1}H and ^{13}C NMR. Compound **13** could also be obtained by hydrazinolysis of **4**. The *O*-amine function of **13** can be readily protected by the Fmoc group, offering the desired compound **14** in 73% yields. This function was also protected with Boc_2O. To obtain an analytical pure sample, the carboxylic acid was protected as methyl ester. However, N-methylation occurred simultaneously, leading to the compound **15**. Finally, coupling of carboxylic acid **4** with oxyamine **12** led successfully to the dinucleoside **16** in 50% overall yield from **10**. Compound **16** has been fully characterized by ^{1}H, ^{13}C NMR and HRMS. Surprisingly, we have observed the formation of phthalimido ring-opening product **17** during column chromatography of **16** with CH_2Cl_2/MeOH (Scheme 2). The slowly ring opening of the phthalimido group of **16** by CD_3OD in the NMR sample has also been observed. It is to be noticed that the instability of phthalimidooxy group in MeOH has never been reported.[22]

We then decided to investigate the stability of uridine derivative **4** in the presence of MeOH (Scheme 3). Treatment of compound **4** with K_2CO_3 in MeOH at rt led to the ring-opening product **18** in 30% isolated yield. Esterification of **4** with MeI and $NaHCO_3$ in DMF led quantitatively to the ester **19**. Deacetylation of **19** with NaOMe led firstly to the phthalimido ring-opening product **20** after 40 min reaction. Compound **20** can be converted back to the phthalimidooxy derivative **19** by heating at 130–140 °C under reduced pressure. Further treatment with NaOMe deprotected the 2′-*O*-acetyl group, providing a mixture of compounds **21** and **22**. Similar phenomena have been observed during the saponification of **19** with K_2CO_3 or NaOH (4 M) in MeOH. The ring closure reaction of the phthalimido group has also been observed by heating **21** under reduced pressure, along with some decomposition. Under acidic condition in MeOH, we have observed successive transformations of compound **4** to the methyl ester **22** through the intermediates **19**, **20** and **21** on TLC.



Scheme 3. Reagents and conditions: (i) K_2CO_3, MeOH, 30%; (ii) MeI, $NaHCO_3$, DMF, 100%; (iii) NaOMe, MeOH, rt, 40 min, 82%; (iv) 30–40 mmHg, 130–140 °C, 2 h; (v) NaOMe, MeOH, rt, overnight; (vi) MeOH, HCl_{concd}.

3. Conclusion

In summary, nucleoside aminooxy acids have been successfully synthesized for the first time by N-glycosylation of sugar aminooxy acids with five common nucleic bases. Fmoc and Boc protected uridine aminooxy esters have also been prepared. Coupling reaction of uridine aminooxy acid derivatives led to the desired *N*-oxy amide-linked dinucleoside. The instability of phthalimidooxy group in methanol has been observed, leading reversibly to the imide ring-opening product. This reaction was catalysed under basic or acid conditions. These nucleoside aminooxy acid derivatives might be useful building blocks for the synthesis of novel oligoribonucleotides mimics as well as conjugates with peptides, carbohydrates or reporter compounds.

4. Experimental

4.1. General

^{1}H and ^{13}C NMR spectra were recorded on a 400 spectrometer in $CDCl_3$, CD_3OD or DMSO-d_6 solutions. Column chromatography was performed on Silica 60 (40–63 μM). Analytical thin-layer chromatography was performed on aluminium percolated plates of Silica Gel 60 F_{254} with detection by UV or by spraying with 6 N H_2SO_4 and heating about 2 min at 300 °C for sugar derivatives. Optical rotations were measured using a Jasco P-2000 polarimeter. High resolution mass spectra (HRMS) were measured by the Service de Spectrométrie de Masse de l'Université Pierre et Marie Curie-Paris 6.

4.2. Preparation of 3-deoxy-1,2-*O*-diacetyl-3-(phthalimidooxymethyl)-D-ribofuranuronic acid 2

To a solution of **1**[13b] (600 mg, 1.65 mmol) in AcOH (21 mL) and Ac_2O (2.1 mL), was added concd H_2SO_4 (100 μL, 1.98 mmol). After stirring overnight, H_2O was added and the mixture extracted with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), filtered and evaporated to give a crude product (633 mg), pure enough for the next steps. Purification by column chromatography (CH_2Cl_2/MeOH/AcOH, 70/1/0.1) afforded **2** (521 mg, 77.3%) as a syrup. R_f=0.35 (CH_2Cl_2/MeOH/AcOH, 6/0.3/0.15); $[\alpha]_D^{20}$ +19.6 (*c* 0.55, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.11 (s, 3H, OAc), 2.22 (s, 3H, OAc), 3.30 (br s, 1H, H-3), 4.52–4.54 (m, 3H, H-4, CH_2), 5.55 (d, 1H, *J*=4.6 Hz, H-2), 6.21 (s, 1H, H-1), 7.70–7.85 (m, 4H, Phth). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.8, 21.1 (CH_3), 43.3 (CH), 73.4 (CH_2), 75.0, 78.1, 99.6 (CH); 123.8 (CH), 128.8 (C), 134.8 (CH), 163.3, 169.9 (C). IR (neat): ν_{max}=1726, 1460 cm^{-1}. HRMS (ESI) calcd for $C_{18}H_{17}NO_{10}$ $[M+Na]^+$: 430.0750; found: 430.0745.

4.3. Preparation of *tert*-butyl 3-deoxy-1,2-*O*-diacetyl-3-(phthalimidooxymethyl)-D-ribofuranuronate 3

To a solution of **2** (146 mg, 0.358 mmol) in CH_2Cl_2 (2.5 mL), were added *t*-BuOH (69 μL, 0.715 mmol), DMAP (18 mg, 0.143 mmol) and DCC (96 mg, 0.468 mmol) in CH_2Cl_2 (0.5 mL) at 0 °C. After stirring overnight, the mixture was filtered and the filtrate diluted with CH_2Cl_2, washed with H_2O, dried ($MgSO_4$), filtered, evaporated to dryness and purified by chromatography (petroleum ether/EtOAc, 4/1) to give **3** (114 mg, 60%) as a colourless syrup. R_f=0.59 (CH_2Cl_2/MeOH, 20/1); mp: 108 °C; $[\alpha]_D^{20}$ +2.4 (*c* 1.61, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.48 (s, 9H, *t*-Bu), 2.07 (s, 3H, OAc), 2.20 (s, 3H, OAc), 3.12–3.22 (m, 1H, H-3), 4.32 (d, 1H, *J*=9.6 Hz, H-4), 4.40–4.50 (m, 2H, CH_2), 5.47 (d, 1H, *J*=5.0 Hz, H-2), 6.15 (s, 1H, H-1), 7.70–7.85 (m, 4H, Phth). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.8, 21.1 (CH_3), 43.4 (CH), 73.5 (CH_2), 75.0, 78.8 (CH); 82.6 (C), 99.6, 123.8 (CH), 128.8 (C), 134.8 (CH), 163.3, 169.2, 169.4, 169.9 (C). IR (neat): ν_{max}=3683, 1796,

1726, 1674 cm^{-1}. HRMS (ESI) calcd for $C_{22}H_{25}NO_{10}$ $[M+Na]^+$: 486.1376; found: 486.1371.

4.4. General procedure for the coupling of 2 with nucleobases

To a mixture of crude **2** (1 mmol, 1 equiv) and nucleobase (1.5 equiv) in anhyd MeCN or $Cl(CH_2)_2Cl$ (15 mL), was added *N,O*-bis(trimethylsilyl)acetamide (BSA, 4.5 equiv). The mixture was then reflux for 30 min until the solution was clear. After cooling to rt, TMSOTf (1.5 equiv) was added, and the mixture reflux again for 2–3 h until the reaction was complete, then cooled to rt. Different treatments have been used for compounds **4**–**8**. Compounds **4**–**6**: the solution was evaporated to dryness; the residue was dissolved in EtOAc and washed with 1 N HCl, brine, dried ($MgSO_4$), filtered, evaporated and purified by column chromatography (CH_2Cl_2/MeOH/AcOH, 40/1/0.1 to 20/1/0.05). Compound **7**: saturated $NaHCO_3$ was added to the reaction mixture, then extracted with $CHCl_3$. The aqueous layer was acidified to pH 4–5 with AcOH, then extracted with $CHCl_3$. The combined organic layers were dried ($MgSO_4$), filtered, evaporated and purified by column chromatography (CH_2Cl_2/MeOH/AcOH, 40/1/0.1 to 25/1/0.07). Compound **8**: saturated $NaHCO_3$ was added to the reaction mixture, then extracted with $CHCl_3$. The organic layer was acidified to pH 4–5 with AcOH, then washed with H_2O, dried ($MgSO_4$), filtered, evaporated and purified by column chromatography (CH_2Cl_2/MeOH/AcOH, 60/1/0.1 to 30/1/0.07).

4.4.1. 2′-O-Acetyl-5′-carboxylic acid-3′-deoxy-3′-(phthalimidooxymethyl)uridine ***4***. Yield 71%. White solid: R_f=0.36 (CH_2Cl_2/MeOH/AcOH, 6/0.9/0.5); $[\alpha]_D^{20}$ +37.1 (*c* 0.24, $CHCl_3$); mp: 154 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.22 (s, 3H, OAc), 3.30–3.40 (m, 1H, H-3′), 4.42 (dd, 1H, *J*=5.0, 9.2 Hz, CH_a-3′), 4.48 (t, 1H, *J*=9.2 Hz, CH_b-3′), 4.73 (d, 1H, *J*=8.2 Hz, H-4′), 5.78 (dd, 1H, *J*=2.8, 6.9 Hz, H-2′), 5.80 (d, 1H, *J*=2.3 Hz, H-1′), 5.81 (d, 1H, *J*=8.2 Hz, H-5), 7.70–7.85 (m, 5H, Phth, H-6), 9.47 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.8 (CH_3), 43.5 (CH), 73.6 (CH_2), 75.3, 79.3, 92.3, 102.6, 123.8 (CH), 128.8 (C); 134.8, 141.2 (CH), 150.2, 163.3, 164.0, 169.7, 173.2, 174.7 (C). IR (neat): ν_{max}=2978, 1726, 1687, 1465 cm^{-1}. HRMS (ESI) calcd for $C_{20}H_{17}N_3O_{10}$ $[M+Na]^+$: 482.0812; found: 482.0806.

4.4.2. 2′-O-Acetyl-5′-carboxylic acid-3′-deoxy-3′-(phthalimidooxymethyl)thymidine ***5***. Yield 70%. Colourless syrup: R_f=0.41 (CH_2Cl_2/MeOH/AcOH, 20/3/0.2); mp: 173 °C; $[\alpha]_D^{20}$ +47.1 (*c* 0.18, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.88 (s, 3H, CH_3), 2.21 (s, 3H, OAc), 3.30–3.40 (m, 1H, H-3′), 4.40–4.50 (m, 2H, CH_2-3′), 4.70 (d, 1H, *J*=6.8 Hz, H-4′), 5.71 (d, 1H, *J*=6.0 Hz, H-2′), 5.76 (s, 1H, H-1′), 7.58 (s, 1H, H-6), 7.70–7.85 (m, 4H, Phth), 9.57 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.5, 20.8 (CH_3), 43.6 (CH), 73.7 (CH_2), 75.6, 79.3, 93.7 (CH), 111.5 (C), 123.8 (CH), 128.8 (C), 134.8 (CH), 137.9 (CH), 150.7, 163.3, 164.4, 170.1, 173.1 (C). IR (neat): ν_{max}=1730, 1700, 1652 cm^{-1}. HRMS (ESI) calcd for $C_{21}H_{19}N_3O_{10}$ $[M+Na]^+$: 496.0968; found: 496.0979.

4.4.3. 2′-O-Acetyl-N-benzoyl-5′-carboxylic acid-3′-deoxy-3′-(phthalimidooxymethyl)cytidine ***6***. Yield 51%. White solid: R_f=0.53 (CH_2Cl_2/MeOH/AcOH, 20/3/0.2); $[\alpha]_D^{20}$ +145.6 (*c* 0.06, MeOH); mp: 143 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.26 (s, 3H, OAc), 3.30–3.40 (m, 1H, H-3′), 4.44 (m, 1H, CH_a-3′), 4.50 (t, 1H, *J*=9.2 Hz, CH_b-3′), 4.78 (d, 1H, *J*=9.2 Hz, H-4′), 5.88 (m, 2H, H-1′,2′), 7.52 (t, 2H, *J*=7.8 Hz, Ph), 7.58–7.60 (m, 1H, H-5), 7.65 (t, 1H, *J*=7.3 Hz, Ph), 7.70–7.85 (m, 4H, Phth), 7.94 (d, 2H, *J*=7.8 Hz, Ph), 8.33 (br s, 1H, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.8 (CH_3), 43.9 (CH), 73.4 (CH_2), 75.8, 80.2, 96.4, 97.6, 123.8, 128.1 (CH), 128.8 (C), 129.1, 133.6, 134.7 (CH), 163.2, 167.1, 169.8, 172.7 (C). IR (neat): ν_{max}=3696, 1735, 1713, 1487 cm^{-1}. HRMS (ESI) calcd for $C_{27}H_{22}N_4O_{10}$ $[M+Na]^+$: 585.1234; found: 585.1228.

4.4.4. 2′-O-Acetyl-N-benzoyl-5′-carboxylic acid-3′-deoxy-3′-(phthalimidooxymethyl)adenosine ***7***. Yield 41%. White solid: R_f=0.44 (CH_2Cl_2/MeOH/AcOH, 20/3/0.2); mp: 146 °C; $[\alpha]_D^{20}$ +15.4 (*c* 0.19, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.35 (s, 3H, OAc), 3.35–3.45 (m, 1H, H-3′), 4.45–4.52 (m, 1H, CH_a-3′), 4.57 (t, 1H, *J*=9.2 Hz, CH_b-3′), 4.75 (d, 1H, *J*=9.6 Hz, H-4′), 5.75 (m, 1H, *J*=5.0 Hz, H-2′), 6.3 (s, 1H, H-1′), 7.52–7.60 (m, 3H, Bz), 7.70–7.85 (m, 4H, Phth), 8.02 (d, 2H, *J*=6.7 Hz, Bz), 8.49 (s, 1H, H-8), 8.78 (s, 1H, 2-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.8 (CH_3), 43.6 (CH), 72.8 (CH_2), 77.3, 80.3, 92.3, 123.9, 128.2 (CH), 128.8 (C), 129.0, 133.2, 134.9, 150.1, 152.3 (CH), 163.3 (C). IR (neat): ν_{max}=3678, 1735, 1604, 1582 cm^{-1}. HRMS (ESI) calcd for $C_{28}H_{22}N_6O_9$ $[M+Na]^+$: 609.1346; found: 609.1352.

4.4.5. 5′-Carboxylic acid-3′-deoxy-2-N,2′-O-diacetyl-6-O-dipenylcarbamoyl-3′-(phthalimidooxymethyl)guanosine ***8***. Yield 47%. White solid: R_f=0.65 (CH_2Cl_2/MeOH/AcOH, 20/3/0.2); mp: 179 °C; $[\alpha]_D^{20}$ +46.3 (*c* 0.20, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 1.99 (s, 3H, OAc), 2.22 (s, 3H, OAc), 4.15–4.25 (m, 1H, H-3′), 4.55–4.70 (m, 3H, CH_2-3′, H-4′), 6.08 (d, 1H, *J*=5.5 Hz, H-2′), 6.33 (s, 1H, H-1′), 7.20–7.55 (m, 10H, $CONPh_2$), 7.78–7.79 (m, 4H, Phth), 8.58 (s, 1H, H-8). ^{13}C NMR (100 MHz, CD_3OD): δ 19.4, 23.2 (CH_3), 43.0 (CH), 73.2 (CH_2), 77.1, 79.7, 90.6, 123.0, 126.5, 126.9, 127.0, 127.4, 127.5 (CH), 128.9 (CH), 129.1 (C), 134.5 (CH), 144.4, 154.0, 163.7, 170.5 (C). IR (neat): ν_{max}=1726, 1626, 1600, 1496 cm^{-1}. HRMS (ESI) calcd for $C_{36}H_{29}N_7O_{11}$ $[M+Na]^+$: 758.1823; found: 758.1823.

4.5. Preparation of 2′-*O*-acetyl-5′-carboxylic acid *tert*-butyl ester-3′-deoxy-3′-(phthalimidooxymethyl)-*N*-*tert*-butyluridine 9 and 2′-*O*-acetyl-5′-carboxylic acid *tert*-butyl ester-3′-deoxy-3′-(phthalimidooxymethyl)uridine 10

To a solution of **4** (264 mg, 0.574 mmol) in anhyd CH_2Cl_2 (9 mL) and cyclohexane(3 mL), was added *t*-BuOC(=NH)CCl_3 (264 μL, 4.47 mmol). After overnight stirring at rt, EtOAc was added to the reaction mixture. The solution was washed with saturated $NaHCO_3$, brine, dried ($MgSO_4$), filtered and condensed under reduced pressure to dryness, purified by chromatography (petroleum ether/EtOAc, 4/1, 2/1 then 1/1) to get compounds **9** (51%) and **10** (42%).

Compound **9**: white solid, R_f=0.45 (CH_2Cl_2/MeOH, 20/1); mp: 76 °C; $[\alpha]_D^{20}$ +85.6 (*c* 0.89, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H, *t*-Bu), 1.64 (s, 9H, *t*-Bu) 2.20 (s, 3H, OAc), 2.90–2.96 (m, 1H, H-3′), 4.28 (dd, 1H, *J*=6.4, 8.7 Hz, CH_a-3′), 4.43 (dd, 1H, *J*=6.8, 8.7 Hz, CH_b-3′), 4.62 (d, 1H, *J*=9.6 Hz, H-4′), 5.74 (dd, 1H, *J*=1.4, 5.5 Hz, H-2′), 5.82 (d, 1H, *J*=7.8 Hz, H-5), 6.02 (d, 1H, *J*=1.8 Hz, H-1′), 7.75–7.85 (m, 4H, Phth), 8.33 (d, 1H, *J*=7.3 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.8, 28.0, 28.3 (CH_3); 44.0 (CH), 73.4 (CH_2), 76.0, 80.4 (CH), 83.5, 83.6 (C), 92.1, 97.8, 123.8 (CH), 128.8 (C), 134.8, 141.9 (CH), 155.4, 163.0, 169.0, 169.6, 171.5 (C). IR (neat): ν_{max}=1734, 1656, 1630, 1526 cm^{-1}. HRMS (ESI) calcd for $C_{28}H_{33}N_3O_{10}$ $[M+Na]^+$: 594.2064; found: 594.2041.

Compound **10**: white solid, R_f=0.33 (CH_2Cl_2/MeOH, 20/1); mp: 108 °C; $[\alpha]_D^{20}$ +63.8 (*c* 0.51, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.50 (s, 9H, *t*-Bu), 2.22 (s, 3H, OAc), 2.98–3.07 (m, 1H, H-3′), 4.34 (dd, 1H, *J*=6.0, 9.2 Hz, CH_a-3′), 4.45 (dd, 1H, *J*=7.3, 9.2 Hz, CH_b-3′), 4.65 (d, 1H, *J*=8.7 Hz, H-4′), 5.67 (dd, 1H, *J*=2.8, 6.4 Hz, H-2′), 5.82 (d, 1H, *J*=2.3, 7.8 Hz, H-5), 6.02 (d, 1H, *J*=2.3 Hz, H-1′), 7.75–7.85 (m, 4H, Phth), 8.15 (d, 1H, *J*=8.3 Hz, H-6), 8.50 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.8, 28.1 (CH_3); 43.9 (CH), 73.3 (CH_2), 75.4, 79.8 (CH), 83.9 (C), 90.8, 102.9, 123.9 (CH), 128.8 (C), 134.9, 140.1 (CH), 150.0, 162.9, 163.2, 169.4, 169.6 (C). IR (neat): ν_{max}=3683, 1735, 1696, 1457 cm^{-1}. HRMS (ESI) calcd for $C_{24}H_{25}N_3O_{10}$ $[M+Na]^+$: 538.1438; found: 538.1432.

4.6. Conversion of compound 9 to 10

To a solution of **9** (7 mg, 0.0122 mmol) in CH_2Cl_2 (0.7 mL), was added AcOH (0.3 mL). After stirring overnight at rt, the solvent was evaporated, the residue dried by oil pump to give quantitatively the compound **10**.

4.7. Preparation of 3′-aminooxymethyl-5′-carboxylic acid *tert*-butyl ester-3′-deoxyuridine 12

To a solution of **10** (15 mg, 0.029 mmol) in MeOH (1 mL), was added $NH_2NH_2{\cdot}H_2O$ (8.3 μL, 0.17 mmol). After 3 h stirring, the mixture was evaporated to dryness at rt, and to the residue was added saturated $NaHCO_3$, then extracted with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), filtered and condensed under reduced pressure to give the titled compound, which was directly used for next steps. An analytical sample was purified by column chromatography (CH_2Cl_2/MeOH, 40/1 to 20/1) to get two products: compounds **12** and 3′-acetamidooxymethyl-5′-carboxylic acid *tert*-butyl ester-3′-deoxyuridine **11**.

Compound **12**: yield 77%, white solid, R_f=0.23 (CH_2Cl_2/MeOH, 20/1); mp: 92 °C; $[\alpha]_D^{20}$ +72.4 (*c* 0.20, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.56 (s, 9H, *t*-Bu), 2.50–2.60 (m, 1H, H-3′), 3.93 (dd, 1H, *J*=5.5, 11.0 Hz, CH_a-3′), 4.09 (dd, 1H, *J*=8.3, 11.0 Hz, CH_b-3′), 4.44 (d, 1H, *J*=10.1 Hz, H-4′), 4.55 (d, 1H, *J*=6.4 Hz, H-2′), 5.73 (d, 1H, *J*=8.2 Hz, H-5), 5.82 (s, 1H, *J*=8.2 Hz, H-1′), 8.44 (d, 1H, *J*=8.2 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.1 (CH_3); 45.6 (CH), 70.5 (CH_2), 75.9, 80.5 (CH), 83.3 (C), 93.8, 102.0, 140.7 (CH); 151.2, 163.9, 170.7 (C). IR (neat): ν_{max}=3674, 1735, 1691, 1452 cm^{-1}. HRMS (ESI) calcd for $C_{14}H_{21}N_3O_7$ $[M+Na]^+$: 366.1277; found: 366.1272.

Compound **11**: yield 8%, white syrup, R_f=0.11 (CH_2Cl_2/MeOH, 20/1); $[\alpha]_D^{20}$ +97.3 (*c* 0.44, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.52 (s, 9H, *t*-Bu), 1.88 (s, 3H, OAc), 2.55–2.65 (m, 1H, H-3′), 4.11–4.17 (m, 1H, CH_a-3′), 4.31 (t, 1H, *J*=9.6 Hz, 1H, CH_b-3′), 4.51 (d, 1H, *J*=8.7 Hz, H-4′), 4.69 (s, 1H, H-2′), 5.57 (br s, 1H, OH), 5.70 (d, 1H, *J*=7.8 Hz, H-5), 5.87 (s, 1H, H-1′), 8.45 (d, 1H, *J*=7.8 Hz, H-6), 9.98 (s, 1H, NH), 10.7 (s, 1H, ONHAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.8, 28.1 (CH_3); 44.6 (CH), 71.2 (CH_2), 75.3, 79.4 (CH), 83.5 (C), 93.2, 101.9, 141.2 (CH), 151.5, 164.1, 169.1, 170.7 (C). IR (neat): ν_{max}=3673, 1930, 1734, 1660, 1456 cm^{-1}. HRMS (ESI) $C_{16}H_{23}N_3O_8$ $[M+Na]^+$: 408.1383; found: 408.1392.

4.8. Preparation of 3′-aminooxymethyl-5′-carboxylic acid-3′-deoxyuridine 13

To a solution of **12** (30 mg, 0.065 mmol) in CH_2Cl_2 (0.5 mL) was added TFA (0.1 mL). After stirring for 4–6 h at rt, the mixture was diluted with EtOAc, evaporated to dryness to afford 19 mg (colourless syrup, 100%) of **13**, pure enough for NMR analysis. R_f=0.34 (CH_2Cl_2/MeOH/AcOH, 6/1/0.1); $[\alpha]_D^{20}$ +68.8 (*c* 0.30, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 2.65–2.75 (m, 1H, H-3′), 4.35 (dd, 1H, *J*=5.0, 9.2 Hz, CH_a-3′), 4.45 (t, 1H, *J*=7.8 Hz, CH_b-3′), 4.50 (d, 1H, *J*=5.5 Hz, H-2′), 4.63 (d, 1H, *J*=9.6 Hz, H-4′), 5.80 (d, 1H, *J*=8.2 Hz, H-5), 5.83 (s, 1H, H-1′), 8.25 (d, 1H, *J*=7.8 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 44.4 (CH), 70.6 (CH_2), 74.5, 79.1, 93.6, 100.8, 140.9 (CH), 150.9, 165.0, 172.7 (C). IR (neat): ν_{max}=3674, 1926, 1683, 1387 cm^{-1}.

4.9. Preparation of 5′-carboxylic acid *tert*-butyl ester-3′-(9*H*-fluoren-9-yl)methoxycarbonylaminooxymethyl-3′-deoxyuridine 14

To a solution of **12** (10 mg, 0.029 mmol) in 1,4-dioxane/H_2O (1/1, 1.3 mL) was added $NaHCO_3$ (7 mg, 0.088 mmol). After stirring for 30 min, FmocOSu (15 mg, 0.044 mmol) was added and the mixture stirred overnight. After addition of H_2O, the mixture was extracted with EtOAc. The organic layer was washed with 1 N HCl, $NaHCO_3$, brine, dried, filtered and evaporated to dryness. The residue was purified by column chromatography (CH_2Cl_2/MeOH, 60/1 to 40/1) to afford **14** (12 mg, 73%) as a white solid, R_f=0.39 (CH_2Cl_2/MeOH, 20/1); mp: 106 °C; $[\alpha]_D^{20}$ +39.8 (*c* 0.59, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.51 (s, 9H, *t*-Bu), 2,60–2.70 (br s, 1H, H-3′), 4.14–4.21 (m, 2H, CH_a-3′, Fmoc–CH), 4.32 (t, 1H, *J*=10.1 Hz, CH_b-3′), 4.45–4.52 (m, 3H, H-4′, Fmoc–CH_2), 4.68 (m, 1H, H-2′), 5.17 (s, 1H, OH), 5.69 (d, 1H, *J*=8.2 Hz, H-5), 5.89 (d, 1H, *J*=1.8 Hz, H-1′), 7.25–7.28 (m, 2H, H–Ar), 7.34–7.39 (m, 2H, H–Ar), 7.54 (d, 1H, *J*=7.3 Hz, H–Ar), 7.55 (d, 1H, *J*=7.3 Hz, H–Ar), 7.72 (d, 1H, *J*=7.3 Hz, H–Ar), 7.73 (d, 1H, *J*=7.3 Hz, H–Ar), 8.43 (d, 1H, *J*=8.2 Hz, H-6), 8.60 (s, 1H, NH), 10.5 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.1 (CH_3), 44.4, 47.0 (CH), 67.7, 71.7 (CH_2), 75.5, 79.6 (CH), 83.5 (C), 93.2, 102.0 (CH), 120.1, 125.0, 127.2, 127.8, 141.0 (CH), 141.4, 143.5, 151.4, 157.8, 163.8, 170.7(C). IR (neat): ν_{max}=3670, 1926, 1691, 1448 cm^{-1}. HRMS (ESI) calcd for $C_{29}H_{31}N_3O_9$ $[M+Na]^+$: 588.1958; found: 588.1971.

4.10. Preparation of 5′-carboxylic acid methyl ester-3′-(*N*-*tert*-butoxycarbonyl-*N*-methyl)aminooxymethyl-3′-deoxyuridine 15

To a solution of **4** (30 mg, 0.065 mmol) in MeOH (1 mL), was added $NH_2NH_2{\cdot}H_2O$ (19 μL, 0.196 mmol). After overnight stirring, the mixture was evaporated to dryness. To this residue in 1,4-dioxane/H_2O (1/1, 3 mL), was added $NaHCO_3$ (32 mg, 0.38 mmol). After stirring for 30 min, Boc_2O (50 mg, 0.23 mmol) was added to the mixture and then stirred overnight. After addition of H_2O, the mixture was extracted with Et_2O. The aqueous layer was acidified to pH 3–4 with AcOH, then extracted with EtOAc. The combined organic layers were washed with brine, dried, filtered and evaporated to dryness. Purification by column chromatography (CH_2Cl_2/MeOH/AcOH, 60/1/0.2 to 40/1/0.2) afforded a mixture. To get a pure product, the mixture was dissolved in DMF (1.5 mL), $NaHCO_3$ (19 mg, 0.23 mmol) and MeI (10 μL, 0.15 mmol) were added. After stirring overnight, the mixture was diluted with EtOAc, washed with H_2O, brine, dried, filtered, concentrated and purified by column chromatography (petroleum ether/EtOAc, 5/5) to give 7 mg (23%) of **15** as a white solid, R_f=0.5 (CH_2Cl_2/MeOH, 20/1); mp: 113 °C; $[\alpha]_D^{20}$ +38.2 (*c* 0.06, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.47 (s, 9H, *t*-Bu), 2.45–2.55 (m, 1H, H-3′), 3.33 (s, 3H, N–Me), 3.83 (s, 3H, CO_2Me), 4.11 (dd, 1H, *J*=8.7, 10.1 Hz, CH_a-3′), 4.19 (dd, 1H, *J*=5.0, 8.7 Hz, 1H, CH_b-3′), 4.59 (m, 2H, H-2′,4′), 5.12 (d, 1H, OH), 5.80 (d, 1H, *J*=8.2 Hz, H-5), 5.93 (d, 1H, *J*=1.4 Hz, H-1′), 7.35 (s, 1H, CONH), 8.09 (d, 1H, *J*=7.8 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.7, 28.3 (CH_3), 44.9 (CH), 52.9 (CH_3), 72.2 (CH_2), 76.4, 77.3, 79.1 (CH); 83.7 (C), 94.4, 101.5, 137.9 (CH), 163.2, 171.7 (C). IR (neat): ν_{max}=3196, 2996, 2848, 1748, 1704, 1652, 1626 cm^{-1}. HRMS (ESI) calcd for $C_{17}H_{25}N_3O_9$ $[M+Na]^+$: 438.1488; found: 438.1483.

4.11. Preparation of dinucleoside 16

To a solution of **4** (30 mg, 0.065 mmol) in anhyd DMF (0.5 mL), were added HOBt (16.5 mg, 0.118 mmol) and EDC (23 mg, 0.118 mmol) under N_2 atmosphere at 0 °C. After 20 min stirring, compound **12** (22 mg, 0.065 mmol) in anhyd DMF (0.5 mL) was added. The reaction mixture was stirred at rt overnight. The solution was diluted with EtOAc, washed with 1 N HCl, saturated $NaHCO_3$, brine, dried, filtered and concentrated to dryness. The residue was dissolved in EtOAc (4 mL), and cyclohexane (6 mL) was added to precipitate the product. Filtration afforded **16** as a white solid (25 mg, 50%), R_f=0.23 (CH_2Cl_2/MeOH, 15/1); mp: 197 °C; $[\alpha]_D^{20}$ +60.7 (*c* 0.71, $CHCl_3$); ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.38 (s, 9H), 2.10 (s, 3H), 2.40–2.50 (s, 1H), 3.20–3.25 (m, 1H), 3.93–3.97 (m, 1H), 4.11 (t, 1H, *J*=10.1 Hz), 4.23–4.27 (m), 1.38 (s, 9H, *t*-Bu), 2.10 (s, 3H, OAc), 2.40–2.50 (s, 1H, H-3′), 3.20–3.25 (m, 1H, H-3′), 3.93–3.97 (m, 1H, CHON), 4.11 (t, 1H, *J*=10.1 Hz, CHON), 4.23–4.27 (m, 1H, CHON), 4.31–4.38 (m, 4H, –CHON–, H-2′, 2×H-4′), 5.59 (d,

1H, J=6.4 Hz, H-2′), 5.66–5.74 (m, 3H, H-1′, 2×H-5′), 5.87 (s, 1H, H-1′), 6.07 (s, 1H, –OH), 7.82–7.89 (m, 4H, Phth), 7.94 (d, 1H, J=7.8 Hz, H-6), 8.08 (d, 1H, J=8.3 Hz, H-6), 11.38 (s, 1H, NH), 11.41 (s, 1H, NH), 11.75 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.0, 27.9 (CH_3); 43.0, 44.7 (CH); 71.6, 73.8 (CH_2); 74.6, 75.8, 78.6, 79.7 (CH), 82.9 (C), 91.4, 93.0, 101.7, 102.5, 123.9 (CH); 129.1(C), 135.4, 140.3, 141.9 (CH), 150.8, 150.9, 163.5, 163.7, 169.8, 170.8 (C). IR (neat): ν_{max}=3674, 1930, 1726, 1687, 1452 cm^{-1}. HRMS (ESI) calcd for $C_{34}H_{36}N_6O_{16}$ [M+Na]$^+$: 807.2085; found: 807.2080.

4.12. Preparation of 5′-carboxylic acid-3′-deoxy-3′-[(methoxycarbonyl)benzamidooxy]methyluridine 18

To a solution of **4** (131 mg, 0.285 mmol) in anhyd MeOH (2.8 mL) was added Na_2CO_3 (13 mg, 0.124 mmol). After stirring overnight, the solution was acidified to pH 1–2 with 4 M HCl, evaporated to dryness at rt. The residue was purified by column chromatography (CH_2Cl_2/MeOH, 50/1 to 40/1) to afford **18** (36 mg, 30%) as a white solid. R_f=0.39 (CH_2Cl_2/MeOH/AcOH, 20/1/0.07); mp: 156 °C; $[\alpha]_D^{20}$ +135.0 (*c* 0.08, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 2.74 (s, 1H, H-3′), 3.87 (s, 3H, CO_2Me), 4.29 (dd, 1H, J=4.6, 9.6 Hz, CH_a-3′), 4.38 (t, 1H, J=9.6 Hz, CH_b-3′), 5.58 (m, 2H, H-2′,4′), 5.66 (d, 1H, J=7.8 Hz, H-5), 5.84 (s, 1H, H-1′), 7.44 (dd, 1H, J=0.9, 7.8 Hz, Ph), 7.56–7.65 (m, 2H, Ph), 7.94–7.96 (m, 1H, Ph), 8.50 (d, 1H, J=7.3 Hz, H-6). ^{13}C NMR (100 MHz, CD_3OD): δ 45.9 (CH), 53.1 (CH_3), 72.4 (CH_2), 76.6, 80.6, 95.0, 101.8, 129.5 (CH); 130.6 (C), 131.3, 131.6, 133.5 (CH), 136.0 (C), 142.5 (CH), 152.2, 166.5, 167.7, 169.7, 174.5 (C). IR (neat): ν_{max}=3670, 1735, 1678, 1452 cm^{-1}. HRMS (ESI) calcd for $C_{19}H_{19}N_3O_{10}$ [M+Na]$^+$: 472.0968; found: 472.0963.

4.13. Preparation of 2′-*O*-acetyl-5′-carboxylic acid methyl ester-3′-deoxy-3′-(phthalimidooxymethyl)uridine 19

To a solution of **4** (30 mg, 0.065 mmol) in DMF (1 mL) were added $NaHCO_3$ (11 mg, 0.13 mmol) and CH_3I (5.3 μL, 0.085 mmol). After stirring overnight, the mixture was diluted with EtOAc, washed with H_2O, brine, dried, filtered and concentrated to dryness to give quantitatively **19**, pure enough for next steps. An analytical sample was purified by column chromatography (CH_2Cl_2/MeOH, 80/1 to 50/1) to give a white solid, R_f=0.51 (CH_2Cl_2/MeOH, 20/1); mp: 116 °C; $[\alpha]_D^{20}$ +52.4 (*c* 0.78, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.20 (s, 3H, OAc), 3.05–3.15 (m, 1H, H-3′), 3.84 (s, 3H, CO_2Me), 4.33 (dd, 1H, J=6.4, 9.2 Hz, CH_a-3′), 4.46 (dd, 1H, J=7.3, 9.2 Hz, CH_b-3′), 4.79 (d, 1H, J=8.2 Hz, H-4′), 5.65 (dd, 1H, J=3.2, 6.4 Hz, H-2′), 5.78 (dd, 1H, J=1.8, 7.8 Hz, H-5), 6.00 (d, 1H, J=2.8 Hz, H-1′), 7.75–7.85 (m, 4H, Phth), 7.98 (d, 1H, J=8.2 Hz, H-6), 8.86 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.7 (CH_3); 43.7 (CH), 53.1 (OCH_3), 73.4 (CH_2), 75.2, 79.1, 90.9, 103.0, 123.8 (CH), 128.8 (C), 134.9, 140.1 (CH), 150.0, 163.2, 169.5, 169.5, 170.9 (C). IR (neat): ν_{max}=3674, 1730, 1691, 1452 cm^{-1}. HRMS (ESI) calcd for $C_{21}H_{19}N_3O_{10}$ [M+Na]$^+$: 496.0968; found: 496.0963.

4.14. Preparation of 2′-*O*-acetyl-5′-carboxylic acid methyl ester-3′-deoxy-3′-[(methoxycarbonyl)benzamidooxy] methyluridine 20

To solution of **19** (30 mg, 0.063 mmol) in anhyd MeOH (1 mL) was added 1.38 M NaOMe (20 μL, 0.276 mmol). After stirring 30–40 min, the solution was acidified to pH 1–2 with 4 M HCl, evaporated to dryness at rt. The residue was purified by column chromatography (CH_2Cl_2/MeOH, 50/1 to 40/1) to afford **20** (28 mg, 81.5%) as colourless oil, R_f=0.33 (CH_2Cl_2/MeOH, 20/1); $[\alpha]_D^{20}$ +43.6 (*c* 0.28, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.15 (s, 3H, OAc), 3.05–3.15 (m, 1H, H-3′), 3.84 (s, 6H, 2× CO_2Me), 4.31 (m, 2H, CH_2-3′), 4.68 (s, 1H, H-4′), 5.62 (m, 2H, H-2′,5), 5.87 (s, 1H, H-1′), 7.41–7.56 (m, 3H, Ph), 7.90 (d, 1H, J=7.4 Hz, Ph), 7.99 (d, 1H, J=7.4 Hz, H-6), 8.91 (s, 1H, NH), 9.29 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.7 (CH_3), 43.3 (CH), 52.7, 53.1 (CH_3), 71.0 (CH_2), 75.9, 79.7, 91.6, 102.4 (CH), 128.5 (C), 128.8, 130.3, 132.5 (CH), 135.2 (C), 140.5 (CH), 150.1, 163.1, 166.4, 169.7, 171.4 (C). IR (neat): ν_{max}=3683, 1717, 1682, 1460 cm^{-1}. HRMS (ESI) calcd for $C_{22}H_{23}N_3O_{11}$ [M+Na]$^+$: 528.1230; found: 528.1225.

4.15. Preparation of 5′-carboxylic acid methyl ester-3′-deoxy-3′-[(methoxycarbonyl)benzamidooxy]methyluridine 21 and 5′-carboxylic acid methyl ester-3′-deoxy-3′-phthalimidooxymethyluridine 22

To a solution of **20** (32 mg, 0.068 mmol) in anhyd MeOH (1 mL) was added 1.38 M NaOMe (10 μL, 0.068 mmol). After stirring overnight, the solution was acidified to pH 1–2 with 4 M HCl, evaporated to dryness at rt, the residue was purified by column chromatography (CH_2Cl_2/MeOH, 50/1 to 35/1) to afford two compounds: **21** (15 mg, 51%) and **22** (4 mg, 15%).

Compound **21**: white solid, R_f=0.22 (CH_2Cl_2/MeOH, 20/1); mp: 103 °C; $[\alpha]_D^{20}$ +92.2 (*c* 0.49, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.78–2.83 (s, 3H, H-3′), 3.86 (s, 6H, 2× CO_2Me), 4.37 (t, 1H, J=10.0 Hz, CH_a-3′), 4.42–4.50 (m, 1H, CH_b-3′), 4.60–4.70 (m, 2H, H-2′,4′), 5.40 (s, 2H, H-5, OH), 5.85 (s, 1H, H-1′), 7.43–7.55 (m, 3H, Ph), 7.89 (d, 1H, J=7.8 Hz, Ph), 8.39 (d, 1H, J=8.2 Hz, H-6), 9.92 (s, 1H, O–NH), 10.9 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 44.0 (CH), 52.7, 52.9 (CH_3), 70.0 (CH_2), 75.3, 78.7, 93.1, 101.4 (CH), 128.8 (C), 129.0, 130.0, 130.2, 132.5 (CH), 135.2 (C), 141.2 (CH), 151.6, 163.7, 166.3, 167.5, 172.3 (C). IR (neat): ν_{max}=3674, 1726, 1687, 1661 cm^{-1}. HRMS (ESI) calcd for $C_{20}H_{21}N_3O_{10}$ [M+Na]$^+$: 486.1125; found: 486.1119.

Compound **22**: white solid, R_f=0.42 (CH_2Cl_2/MeOH, 20/1); mp: 109 °C; $[\alpha]_D^{20}$ +70.2 (*c* 0.08, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65–2.75 (m, 1H, H-3′), 3.86 (s, 3H, CO_2Me), 4.53 (m, 2H, CH_2-3′), 4.66 (d, 1H, J=10.1 Hz, H-4′), 4.77 (t, 1H, J=4.6 Hz, H-2′), 4.81 (d, 1H, J=4.6 Hz, OH), 5.78 (dd, 1H, J=2.3, 8.2 Hz, H-5), 5.95 (s, 1H, H-1′), 7.75–7.85 (m, 4H, Phth), 8.26 (d, 1H, J=8.2 Hz, H-6), 9.03 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 44.9 (CH), 53.1 (CH_3), 73.7 (CH_2), 75.9, 79.1, 94.1, 102.2, 124.1 (CH); 128.7 (C), 135.0, 140.1 (CH), 150.6, 163.2, 163.7, 171.3 (C). IR (neat): ν_{max}=3670, 1739, 1674, 1456 cm^{-1}. HRMS (ESI) calcd for $C_{19}H_{17}N_3O_9$ [M+Na]$^+$: 454.0862; found: 454.0857.

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