Tetrahedron xxx (xxxx) xxx

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Post-synthetic modification of oligonucleotides containing 5-mono- and 5-di-fluoromethyluridines

Yuta Ito, Honoka Hayashi, Yasufumi Fuchi, Yoshiyuki Hari*

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Nishihama, Yamashiro-cho, Tokushima, 770-8514, Japan

ARTICLE INFO

Article history:
Received 11 October 2020
Received in revised form
9 November 2020
Accepted 12 November 2020
Available online xxx

Keywords:
2′-Deoxy-5-difluoromethyluridine
2′-Deoxy-5-fluoromethyluridine
Nucleoside
Oligonucleotide
Post-synthetic modification

ABSTRACT

Synthesis of 2′-deoxy-5-fluoromethyluridine (dU^{CH2F}) and 2′-deoxy-5-difluoromethyluridine (dU^{CHF2}) phosphoramidites was achieved. The obtained phosphoramidites were efficiently incorporated into oligonucleotides (ONs), and their post-synthetic modification was examined. In the case of dU^{CH2F}-containing ONs, substitution of the fluorine atom with methoxy, hydroxy and amine groups was successful. Moreover, the difluoromethyl group of dU^{CHF2} in the ONs could be converted to a formyl, oxime, or hydrazone by treatment with the corresponding nucleophile.

1. Introduction

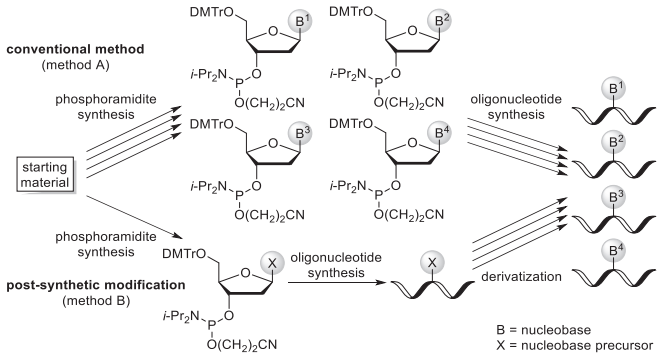
The C5 position of pyrimidine bases in oligonucleotides (ONs) is an attractive site for modification because the substituent at the C5 position does not interfere with Watson–Crick base pairing [1] and RNase H activity [2]. Therefore, ONs containing C5-modified pyrimidine bases have been investigated for application as DNA probes [3] and in antisense technology [4]. In general, ONs containing C5-modified pyrimidine bases have been synthesized using the corresponding modified phosphoramidite monomers on an automated DNA synthesizer (method A in Scheme 1) [5]. However, to synthesize a variety of C5-modified ONs, each modified phosphoramidite monomer needs to be prepared over multiple steps and then introduced onto the ONs, which is time-consuming and inefficient.

Therefore, post-synthetic ON modification is a practical approach for overcoming such drawbacks [6]. As this method allows for the synthesis of various base-modified ONs from a single ON containing a reactive site, it is not necessary to prepare each modified phosphoramidite monomer (method B in Scheme 1). Therefore, the strategy represents a powerful means for the effective exploration of functional ONs. However, the scope of precursors for the preparation of pyrimidine C5-derivatives is extremely limited because such compounds must tolerate the various conditions applied in DNA synthesis. 5-Iodouridine and 2′-deoxy-5-iodouridine have been widely studied as post-synthetic precursors for cross coupling reactions, such as Suzuki-Miyaura [7a,b,c], Sonogashira [8], Stille-Migita [9], and Buchwald-Hartwig [10] reactions, allowing diverse functionalization of C5 position of pyrimidine bases. Although pyrimidine bases with alkyne [11] and alkene [12] derivatives at C5 position were also available, these precursors were primarily used for the introduction of labeling or probe reagents. Recently, Leszczynska et al. reported the post-synthetic construction of 5-(substituted methyl)uridines from 5-(pivaloylmethyl)uridine via nucleophilic substitution with amines and cyanide (Scheme 2a) [13]. Very recently, our group has focused on a unique property of the trifluoromethyl group, and developed the post-synthetic modification of ONs containing 2′-deoxy-5-trifluoromethyluridine (dU^{CF3}) and 2′-deoxy-5-trifluoromethylcytidine (dC^{CF3}) using hydroxide ion and amines as nucleophiles, producing ONs bearing carboxylic acid equivalents at the C5 position (Scheme 2b) [14]. However, a new post-synthetic method for modification at the C5 position of pyrimidine bases is still in demand.

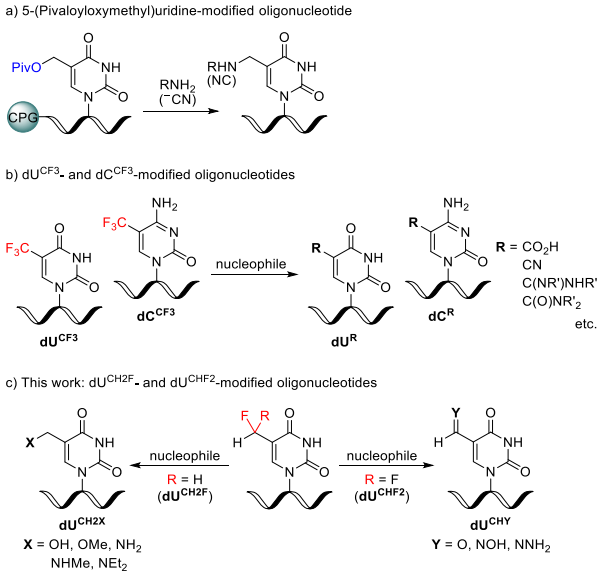
Within this context, we envisaged that 2′-deoxy-5-fluoromethyluridine (dU^{CH2F}) and 2′-deoxy-5-difluoromethyluridine (dU^{CHF2}) could be used as precursors for post-synthetic modification (Scheme 2c). Several examples of base-

* Corresponding author.
E-mail address: hari@ph.bunri-u.ac.jp (Y. Hari).

https://doi.org/10.1016/j.tet.2020.131769
0040-4020/



Scheme 1. Conventional method and post-synthetic modification for the solid-phase oligonucleotide synthesis.



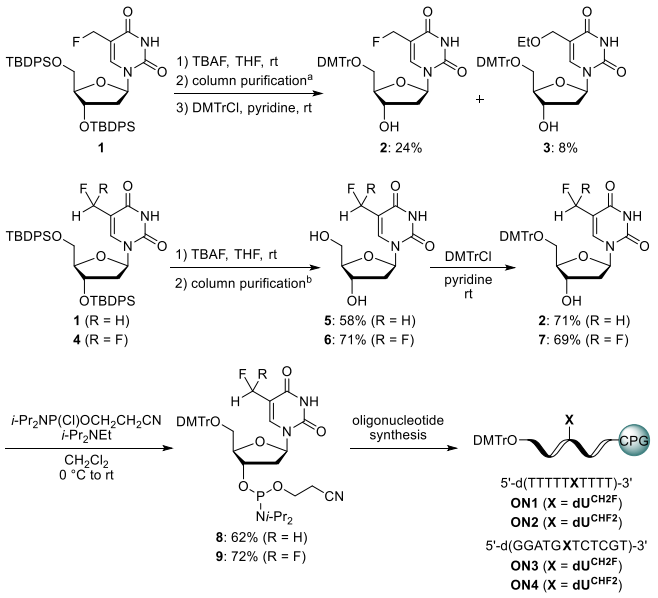
Scheme 2. Post-synthetic diverse functionalization of pyrimidine bases at the C5 position.

mediated substitution reactions of fluorine atoms in dU^{CH2F} monomer derivatives [15] and 5-difluoromethyluracil [16] exist; however, the reactivity of dU^{CH2F} and dU^{CHF2} introduced onto ONs has never been investigated. Herein, we report the post-synthetic modification of dU^{CH2F}- and dU^{CHF2}-modified ONs using various nucleophiles for the preparation of ONs with a variety of 5-substituted uracil bases. Among the C5-modified uracil bases obtained in this method, 2′-deoxy-5-hydroxymethyluridine (dU^{CH2OH}) and 2′-deoxy-5-formyluridine (dU^{CHO}) are considered to be epigenetic bases [17]. Moreover, photocaging of dU^{CH2OH} has been applied for switching transcription [18]. Therefore, developing a practical and operationally simple method to supply ONs containing dU^{CH2OH} and dU^{CHO} is highly desirable for elucidating their biological functionality.

2. Results and discussion

2.1. Synthesis of dU^{CH2F}- and dU^{CHF2}-modified ONs

The synthesis of dU^{CH2F} phosphoramidite commenced from the known 3′-*O*-,5′-*O*-bis-TBDPS-protected dU^{CH2F} **1** (Scheme 3) [19]. Although the deprotection of the TBDPS groups of **1** followed by



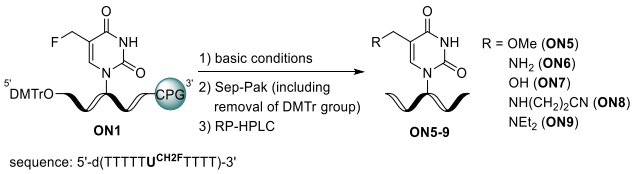
Scheme 3. Synthesis of controlled pore glass (CPG)-attached ONs containing dU^{CH2F} and dU^{CHF2}. [a] Using untreated $CHCl_3$ as eluent. [b] Using $CHCl_3$ treated with molecular sieves 4 Å as eluent.

4,4′-dimethoxytrityl (DMTr) protection of the 5′-hydroxyl group gave desired 5-fluoromethyl product **2**, the yield was low (24%) and the 5-ethoxymethyl congener **3** was obtained as a byproduct. It was considered that the unexpected formation of **3** was caused by ethanol present as a stabilizer in $CHCl_3$, used as an eluent in column chromatography. Thus, when $CHCl_3$ was treated with molecular sieves 4 Å prior to use as an eluent for chromatographic purification, the desired product **5** was obtained in 58% yield without the production of ethoxy-substituted **3**. The instability of the 5-fluoromethyluracil moiety is likely to be problematic for the synthesis of dU^{CH2F}-containing ONs. However, the DMTr protection of the 5′-hydroxyl group of **5** followed by the phosphitylation of the 3′-hydroxyl group successfully afforded the desired dU^{CH2F} phosphoramidite **8**. dU^{CHF2} Phosphoramidite **9** was also obtained from known **4** [19] using the above-described procedure. Phosphoramidites **8** and **9** could be incorporated into oligonucleotides using an automated DNA synthesizer, applying common phosphoramidite chemistry, and the coupling yields of dU^{CH2F} and dU^{CHF2} were estimated to be above 95% by the trityl monitor.

2.2. Post-synthetic modification of dU^{CH2F}-modified ON

Initially, the controlled pore glass (CPG)-attached and 5′-DMTr-protected **ON1**, constituted by a 5′-d (TTTTT**U**^{CH2F}TTTT)-3′ sequence, were subjected to mild deprotection conditions (50 mM K_2CO_3 in MeOH, room temperature, 4 h). The crude product was subsequently purified using Sep-Pak®, followed by reversed-phase HPLC (RP-HPLC) to give **ON5**, bearing a 5-methoxymethyl moiety, in 33% yield, while the dU^{CH2F}-containing ON was not obtained (entry 1 in Table 1 and Fig. 1a). Although the reaction time was reduced from 4 h to 1 h, the dU^{CH2F}-containing ON was not isolated (entry 2 in Table 1 and Fig. 1b). These results suggested that the conversion of the fluoromethyl group was concomitant with ON cleavage from the CPG resin and its deprotection. Treatment of **ON1** under alternative mild deprotection conditions (28% NH_3 aq., room temperature, 2 h) afforded 5-aminomethyl **ON6** in 29% yield along with an inseparable mixture of ONs bearing 5-hydroxymethyl (**ON7**) and 5-[*N*-(2-

Table 1
Post-synthetic modification of **ON1** under basic conditions.



Entry	Basic conditions	Isolated yield[a] (%)
1	50 mM K_2CO_3 in MeOH, rt, 4 h	33 (**ON5**)
2	50 mM K_2CO_3 in MeOH, rt, 1 h	21 (**ON5**)
3	28% NH_3 aq., rt, 2 h	29 (**ON6**) and 9 (**ON7** and **ON8**)
4	20% Et_2NH in MeCN, rt, 1 h then 28% NH_3 aq., rt, 2 h	37 (**ON6**), 5 (**ON7**) and 6 (**ON9**)

[a] Calculated from the CPG resin (0.2 μmol) used for oligonucleotide synthesis.

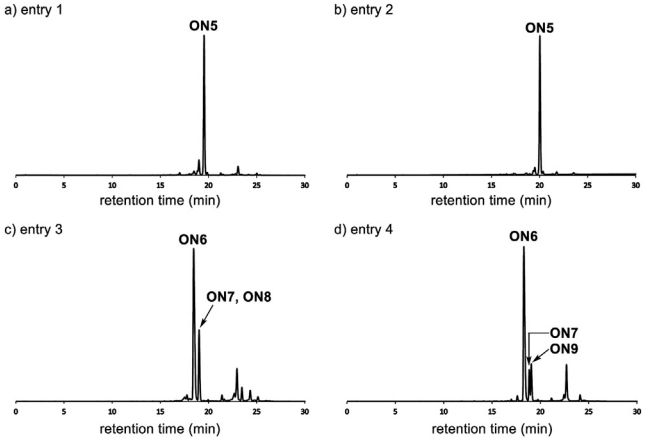


Fig. 1. RP-HPLC chromatograms of crude mixtures of the reactions detailed in Table 1. HPLC conditions: a linear gradient of 5–15% MeCN in 0.1 M triethylammonium acetate (TEAA) buffer (pH 7.0) for 30 min.

cyanoethyl)aminomethyl] groups (**ON8**) in 9% yield, as characterized by electrospray ionization time-of-flight (ESI-TOF) mass spectrometry (entry 3 in Table 1 and Fig. 1c). Treatment with 20% Et_2NH in MeCN selectively deprotected the cyanoethyl groups on the ON phosphate backbone and did not result in detachment from the CPG resin [20]. Thus, to prevent the formation of **ON8,** occurring via the addition of acrylonitrile to **ON6**, treatment of **ON1** with 20% Et_2NH in MeCN followed by cleavage from CPG and conversion of the fluoromethyl group were performed, applying the conditions of 28% NH_3 aq., room temperature, and a reaction time of 2 h. As a result, **ON6** was obtained in 37% yield, without production of **ON8**, although small amounts of **ON7** and 5-diethylaminomethyl **ON9** were formed (entry 4 in Table 1 and Fig. 1d).

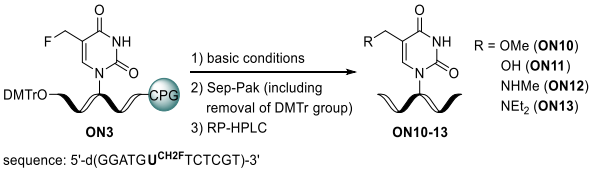
Next, the post-synthetic modification of dU^{CH2F}-modified **ON3**, constituted by a sequence that contains all four DNA bases, was examined using various nucleophiles. The reaction of **ON3** with 50 mM K_2CO_3 in MeOH and 0.1 M NaOH aqueous solution efficiently proceeded to give 5-methoxymethyl-bearing **ON10** (entry 1 in Table 2 and Fig. 2a) and **ON11** containing dU^{CH2OH} (entry 2 in Table 2 and Fig. 2b), respectively. Using $MeNH_2$ as a primary amine, substitution of the fluorine atom to a methylamino group afforded **ON12** (entry 3 in Table 2 and Fig. 2c). Substitution with secondary Et_2NH was sluggish at room temperature, thus the reaction was performed at 60 °C to afford 5-diethylaminomethyl-bearing **ON13** in 41% yield (entry 4 in Table 2 and Fig. 2d).

2.3. Post-synthetic modification of dU^{CHF2}-modified ON

In contrast to dU^{CH2F}-modified ON, dU^{CHF2}-modified ON can be isolated. When CPG-attached **ON2** was subjected to condition A, difluoromethyl-bearing **ON14** was obtained in 41% yield along with **ON15** containing dU^{CHO} in 8% yield (Scheme 4 and Fig. 3). On the other hand, under condition B, **ON15** was afforded as a major product along with **ON14** in 9% yield (Scheme 4 and Fig. 3). Therefore, it was revealed that dU^{CHF2}-containing ON was more stable than its dU^{CH2F} counterpart.

Analogous to the assembly of **ON4** shown in Scheme 4, **ON16**,

Table 2
Post-synthetic modification of **ON3** under basic conditions.



Entry	Basic conditions	Isolated yield[a] (%)
1	50 mM K_2CO_3 in MeOH, rt, 24 h	37 (**ON10**)
2	0.1 M NaOH aq., rt, 24 h	19 (**ON11**)
3	40% $MeNH_2$ aq., rt, 2 h	39 (**ON12**)
4	20% Et_2NH in MeCN, 60 °C, 2 h then 28% NH_3 aq., rt, 24 h	41 (**ON13**)

[a] Calculated from the CPG resin (0.2 μmol) used for oligonucleotide synthesis.

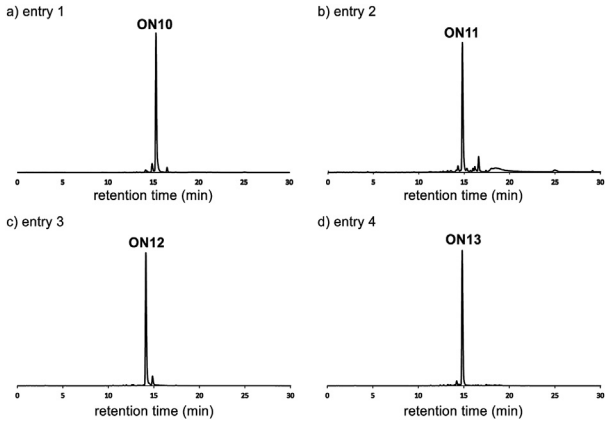
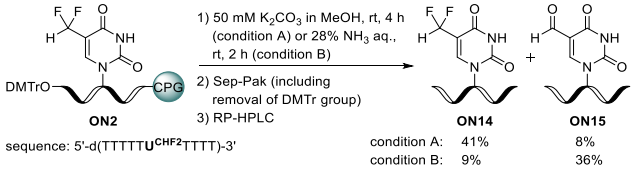


Fig. 2. RP-HPLC chromatograms of crude mixtures of the reactions detailed in Table 2. HPLC conditions: a linear gradient of 5–15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.



Scheme 4. Post-synthetic modification of **ON2** under basic conditions.

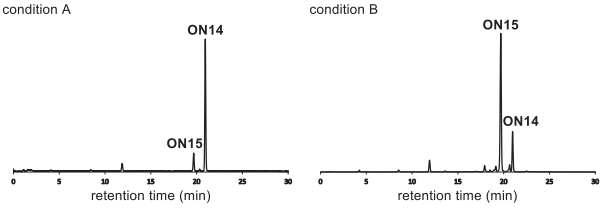


Fig. 3. RP-HPLC chromatograms of crude mixtures of the reactions detailed in Scheme 4. HPLC conditions: a linear gradient of 5–15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.

synthesized via a mixed sequence, was isolated and its post-synthetic modification was explored. Treatment with 28% NH_3 aqueous solution at room temperature was relatively inefficient for the production of **ON17** containing dU^{CHO} (data not shown), likely

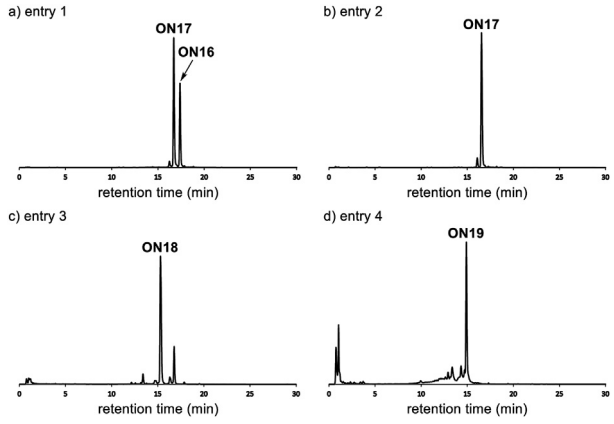


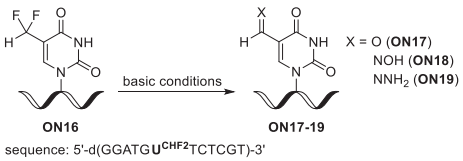
Fig. 4. RP-HPLC chromatograms of crude mixtures of reactions detailed in Table 3. HPLC conditions: a linear gradient of 5–15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min.

as a result of the instability of the 5-formyluracil moiety under alkaline conditions [21,22]. Interestingly, although **ON16** was not consumed when reacted with 50 mM K_2CO_3 in MeOH at room temperature for 24 h (entry 1 in Table 3, and Fig. 4a), increasing the reaction temperature to 60 °C successfully afforded 5-formyl **ON17** as the sole product with complete consumption of the substrate **ON16** (entry 2 in Table 3, and Fig. 4b). Moreover, when treated with NH_2OH and NH_2NH_2 as nucleophiles, the difluoromethyl group was converted to an oxime (**ON18**) and a hydrazone (**ON19**), respectively (entries 3 and 4 in Table 3, and Fig. 4c and 4d).

3. Conclusions

In this study, dU^{CH2F} and dU^{CHF2} phosphoramidites were synthesized and incorporated into ONs. The straightforward and diverse functionalization of uracil bases at the C5 position of ONs was achieved by post-synthetic modification of dU^{CH2F}- and dU^{CHF2}-bearing ONs. In particular, as dU^{CHO} is not only known as a thymidine lesion in DNA [23a,b,c], but may also act as an epigenetic base for regulating gene transcription, the synthesis of dU^{CHO}-bearing ONs is essential for exploration of dU^{CHO} properties. To date, the formyl precursors, namely aminal [22] and 1,2-dihydroxyethyl units [24], were reported to avoid the decomposition of the 5-formyluracil moiety. The implementation of dU^{CHF2} derivatization developed in this study would allow for the facile preparation of dU^{CHO}-bearing ONs, as it was demonstrated that dU^{CHF2} phosphoramidite could be used as a new precursor of dU^{CHO}

Table 3
Post-synthetic modification of **ON16** under basic conditions.



Entry	Basic conditions	Isolated yield (%)
1[a]	50 mM K_2CO_3 in MeOH, rt, 24 h	37 (**ON17**)
2	50 mM K_2CO_3 in MeOH, 60 °C, 24 h	70 (**ON17**)
3	0.1 M NH_2OH·HCl in 0.1 M phosphate buffer (pH 8.0), rt, 24 h	27 (**ON18**)
4	0.1 M NH_2NH_2 aq., 60 °C, 6 h	29 (**ON19**)

[a] Starting material **ON16** was recovered in 21% yield.

in oligonucleotide synthesis.

4. Experimental

4.1. General

All moisture-sensitive reactions were conducted in well-dried glassware under Ar atmosphere. Anhydrous CH_2Cl_2, pyridine and THF were used as purchased. $CHCl_3$ was treated with molecular sieves 4 Å prior to use. ^{1}H NMR, ^{13}C{^{1}H} NMR, ^{19}F{^{1}H} NMR, ^{31}P{^{1}H} NMR spectra were recorded on a Bruker AVANCE III HD 500 equipped with a BBO cryoprobe or Agilent 400-MR. Chemical shift values were reported in ppm, relative to internal tetramethylsilane (δ = 0.00 ppm) or solvent residual signals (δ = 2.50 ppm for DMSO-d_6) for ^{1}H NMR, solvent residual signals (δ = 77.0 ppm for $CDCl_3$ and δ = 39.5 ppm for DMSO-d_6) for ^{13}C{^{1}H} NMR, internal hexafluorobenzene (δ = 0.00 ppm) for ^{19}F{^{1}H} NMR, and external 5% H_3PO_4 (δ = 0.00 ppm) for ^{31}P{^{1}H} NMR. IR spectra were recorded on a JASCO FT/IR-4200 spectrometer. High-resolution mass spectrometry was performed on a Waters SYNAPT G2-Si (Quadrupole/TOF). For column chromatography, silica gel PSQ-60B (Fuji Silysia) was used. Reaction progress was monitored by analytical thin-layer chromatography (TLC) on pre-coated aluminum sheets (Silica gel 60 F_{254} by Merck). For HPLC, a JASCO EXTREMA (PU-4180, CO-4060 (or CO-4061), UV-4075, and AS-4050) instrument with a CHF122SC (ADVANTEC) fraction collector was used. UV measurements were performed using a JASCO V-730 UV/VIS spectrophotometer to calculate ON amounts. Oligonucleotide synthesis was performed on an automated DNA synthesizer (Gene Design nS-8II).

4.2. Synthesis of phosphoramidites ***8*** and ***9***

4.2.1. 2′-Deoxy-5-fluoromethyluridine (***5***)

To a solution of compound **1** (750 mg, 1.02 mmol) in THF (10 mL), TBAF (1 M in THF, 4.1 mL, 4.1 mmol) was added at room temperature under Ar atmosphere. After being stirred for 0.5 h, the reaction mixture was concentrated *in vacuo*. The crude residue was purified by column chromatography ($CHCl_3$:MeCN = 1:2) to give compound **5** as a white foam (154 mg, 58%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.55 (s, 1H), 8.19 (d, J = 4.0 Hz, 1H), 6.13 (dd, J = 7.0, 6.5 Hz, 1H), 5.27 (d, J = 4.0 Hz, 1H), 5.08 (t, J = 5.0 Hz, 1H), 5.04 (d, J = 49.0 Hz, 2H), 4.26–4.21 (m, 1H), 3.80–3.77 (m, 1H), 3.61 (ddd, J = 12.0, 5.0, 4.0 Hz, 1H), 3.55 (ddd, J = 12.0, 5.0, 4.0 Hz, 1H), 2.14–2.10 (m, 2H). ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6): δ 162.5, 150.3, 142.4 (d, J = 6.0 Hz), 108.7 (d, J = 18.5 Hz), 87.5, 84.4, 77.6 (d, J = 62.0 Hz), 70.1, 61.1, 39.8.^{19}F NMR (376 MHz, DMSO-d_6): δ −39.3 (td, J = 49.0, 4.0 Hz). IR (ATR) cm^{-1}: 3369, 3181, 3061, 2922, 1721, 1660, 1475, 1281. HRMS (ESI-TOF): calcd for $C_{10}H_{13}FN_2NaO_5$ [M + Na]$^+$ 283.0706, found 283.0708.

4.2.2. 2′-Deoxy-5′-O-(4,4′-dimethoxytrityl)-5-fluoromethyluridine (***2***)

To a solution of compound **5** (142 mg, 0.55 mmol) in pyridine (5 mL), DMTrCl (222 mg, 0.66 mmol) was added at room temperature under Ar atmosphere. After being stirred for 19.5 h, the reaction was diluted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue was purified by column chromatography (hexane:AcOEt = 1:3) to give compound **2** as a white foam (217 mg, 71%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.91 (br s, 1H), 7.96 (d, J = 3.5 Hz, 1H), 7.38 (d, J = 7.5 Hz, 2H), 7.31–7.22 (m, 7H), 6.84 (d, J = 9.0 Hz, 4H), 6.37 (dd, J = 7.0, 6.0 Hz, 1H), 4.63 (dd, J = 49.0, 10.5 Hz, 1H), 4.61–4.57 (m, 1H), 4.47 (dd, J = 49.0, 10.5 Hz, 1H), 4.09–4.07 (m, 1H), 3.78 (s, 6H), 3.46 (dd, J = 10.5, 3.0 Hz, 1H), 3.40 (dd, J = 10.5, 3.0 Hz, 1H), 2.48 (ddd, J = 13.5, 6.0, 3.0 Hz, 1H), 2.38–2.29 (m, 2H). ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$): δ 162.3 (d, J = 5.0 Hz), 158.7 (d, J = 2.0 Hz), 150.2 (d, J = 4.0 Hz), 144.2, 140.9 (d, J = 7.0 Hz), 135.3, 135.2, 130.0, 130.0, 128.1, 128.0, 127.2, 113.3, 110.2 (d, J = 19.0 Hz), 87.0, 86.4, 85.2, 77.2 (d, J = 67.5 Hz), 72.2, 63.4, 55.2, 41.2.^{19}F NMR (376 MHz, $CDCl_3$): δ −44.9 (td, J = 48.5, 3.5 Hz). IR (ATR) cm^{-1}: 3414, 3200, 3061, 3016, 2954, 2933, 2837, 1678, 1606, 1508, 1465, 1249. HRMS (ESI-TOF): calcd for $C_{31}H_{31}FN_2NaO_7$ [M + Na]$^+$ 585.2013, found 585.2014.

4.2.3. 3′-O-[2-Cyanoethoxy(diisopropylamino)phosphino]-2′-deoxy-5′-O-(4,4′-dimethoxytrityl)-5-fluoromethyluridine (***8***)

To a solution of compound **2** (199 mg, 0.35 mmol) and *i*-Pr_2NEt (0.31 mL, 1.77 mmol) in CH_2Cl_2 (4.0 mL), *i*-$Pr_2NP(Cl)OCH_2CH_2CN$ (0.12 mL, 0.53 mmol) was added at 0 °C under Ar atmosphere. After being stirred at room temperature for 3 h, the reaction was diluted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue was purified by column chromatography (hexane:AcOEt = 1:1) to give compound **8** as a white foam (167 mg, 63%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.70 (br s, 1H), 8.03 (d, J = 3.5 Hz, 0.5H), 7.99 (d, J = 3.5 Hz, 0.5H), 7.40 (d, J = 7.5 Hz, 1H), 7.39 (d, J = 7.5 Hz, 1H), 7.32–7.23 (m, 7H), 6.85 (d, J = 9.0 Hz, 2H), 6.84 (d, J = 9.0 Hz, 2H), 6.38–6.34 (m, 1H), 4.71–4.65 (m, 1H), 4.61 (dd, J = 48.0, 11.0 Hz, 0.5H), 4.59 (dd, J = 48.0, 11.5 Hz, 0.5H), 4.40 (dd, J = 48.5, 10.5 Hz, 0.5H), 4.39 (dd, J = 48.0, 10.5 Hz, 0.5H), 4.20 (d, J = 3.0 Hz, 0.5H), 4.15 (d, J = 3.0 Hz, 0.5H), 3.88–3.72 (m, 1H), 3.79 (s, 3H), 3.79 (s, 3H), 3.68–3.45 (m, 4H), 3.37 (t, J = 10.5 Hz, 0.5H), 3.36 (t, J = 10.5, 0.5H), 2.63 (t, J = 6.5 Hz, 1H), 2.61 (ddd, J = 13.5, 6.0, 3.0 Hz, 0.5H), 2.54 (ddd, J = 13.5, 5.5, 3.5 Hz, 0.5H), 2.44 (t, J = 6.5 Hz, 1H), 2.37–2.32 (m, 1H), 1.19–1.16 (9H, m), 1.07 (d, J = 7.0 Hz, 3H). ^{19}F NMR (376 MHz, $CDCl_3$): δ −44.5 (td, J = 48.5, 3.5 Hz), −44.7 (td, J = 48.5, 3.5 Hz). ^{31}P NMR (162 MHz, $CDCl_3$): δ 148.9, 148.5. HRMS (ESI-TOF): calcd for $C_{40}H_{48}FN_4NaO_8P$ [M + Na]$^+$ 785.3091, found 785.3091.

4.2.4. 2′-Deoxy-5-difluoromethyluridine (***6***)

To a solution of compound **4** (1.0 g, 1.32 mmol) in THF (7.5 mL), TBAF (1 M in THF, 3.8 mL, 3.8 mmol) was added at room temperature under Ar atmosphere. After being stirred for 0.5 h, the reaction mixture was concentrated *in vacuo*. The crude residue was purified by column chromatography ($CHCl_3$:MeCN = 1:1) to give compound **6** as a white foam (262 mg, 71%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.68 (s, 1H), 8.38 (s, 1H), 6.70 (t, J = 54.5 Hz, 1H), 6.12 (t, J = 6.5 Hz, 1H), 5.25 (d, J = 4.5 Hz, 1H), 5.10 (dd, J = 5.0, 4.5 Hz, 1H), 4.26–4.22 (m, 1H), 3.83–3.81 (m, 1H), 3.62 (ddd, J = 12.0, 4.5, 4.0 Hz, 1H), 3.57 (ddd, J = 12.0, 4.0, 3.5 Hz, 1H), 2.20–2.10 (m, 2H). ^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6): δ 160.7 (t, J = 3.5 Hz), 149.8, 140.6 (t, J = 8.0 Hz), 111.9 (t, J = 235.0 Hz), 107.0 (t, J = 23.0 Hz), 87.6, 84.9, 70.0, 60.8, 40.3.^{19}F NMR (376 MHz, DMSO-d_6): δ 47.1 (dd, J = 293.0, 55.0 Hz), 47.0 (dd, J = 293.0, 55.0 Hz). IR (ATR) cm^{-1}: 3354, 3062, 2924, 1726, 1670, 1483, 1417, 1404, 1299. HRMS (ESI-TOF): calcd for $C_{10}H_{12}F_2N_2NaO_5$ [M + Na]$^+$ 301.0612, found 301.0619.

4.2.5. 2′-Deoxy-5′-O-(4,4′-dimethoxytrityl)-5-difluoromethyluridine (***7***)

To a solution of compound **6** (245 mg, 0.88 mmol) in pyridine (5 mL), DMTrCl (448 mg, 1.32 mmol) was added at room temperature under Ar atmosphere. After being stirred for 13 h, the reaction was diluted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue was purified by column chromatography (hexane:AcOEt = 1:3) to give compound **7** as a white foam (354 mg, 69%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.33 (s, 1H), 8.00 (s, 1H), 7.38 (d, J = 7.5 Hz, 2H), 7.29–7.26 (m, 7H), 7.21 (t, J = 7.5 Hz, 1H), 6.83 (d, J = 9.0 Hz, 4H), 6.33 (t, J = 54.5 Hz, 1H), 6.27 (dd, J = 7.0, 6.0 Hz, 1H),

4.46–4.44 (m, 1H), 4.11–4.09 (m, 1H), 3.78 (s, 6H), 3.40 (dd, J = 10.5, 3.5 Hz, 1H), 3.37 (dd, J = 10.5, 3.5 Hz, 1H), 2.51 (ddd, J = 13.5, 6.0, 3.0 Hz, 1H), 2.48 (br s, 1H), 2.21 (ddd, J = 13.5, 7.0, 6.5 Hz, 1H). ^{13}C {^{1}H} NMR (125 MHz, $CDCl_3$): δ 160.6 (t, J = 4.0 Hz), 158.6, 149.6, 144.3, 139.5 (t, J = 7.0 Hz), 135.3, 130.0, 128.0, 128.0, 127.1, 113.3, 110.3 (t, J = 237.5 Hz), 108.8 (t, J = 24.0 Hz), 87.0, 86.4, 85.8, 72.3, 63.3, 55.2, 41.2.^{19}F NMR (376 MHz, $CDCl_3$): δ 45.9 (dd, J = 297.0, 55.0 Hz), 44.3 (dd, J = 297.0, 55.0 Hz). IR (ATR) cm^{-1}: 3454, 3056, 2931, 2836, 1685, 1607, 1508, 1468, 1248. HRMS (ESI-TOF): calcd for $C_{31}H_{30}F_2N_2NaO_7$ $[M + Na]^+$ 603.1919, found 603.1917.

4.2.6. 3′-O-[2-Cyanoethoxy(diisopropylamino)phosphino]-2′-deoxy-5′-O-(4,4′-dimethoxytrityl)-5-difluoromethyluridine (**9**)

To a solution of compound **7** (100 mg, 0.17 mmol) and *i*-Pr_2NEt (0.15 mL, 0.85 mmol) in CH_2Cl_2 (2.0 mL), *i*-$Pr_2NP(Cl)OCH_2CH_2CN$ (58.0 μL, 0.26 mmol) was added at 0 °C under Ar atmosphere. After being stirred at room temperature for 3 h, the reaction was diluted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue was purified by column chromatography (hexane:AcOEt = 3:2) to give compound **9** as a white foam (95 mg, 72%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.71 (br s, 1H), 8.07 (s, 0.4H), 8.03 (s, 0.6H), 7.40–7.38 (m, 2H), 7.31–7.20 (m, 7H), 6.85–6.82 (m, 4H), 6.29–6.25 (m, 1H), 6.28 (t, J = 54.5 Hz, 0.6H), 6.28 (t, J = 54.5 Hz, 0.4H), 4.58–4.53 (m, 1H), 4.23–4.18 (m, 1H), 3.87–3.29 (m, 6H), 3.79 (s, 3H), 3.79 (s, 3H), 2.67–2.54 (m, 1H), 2.62 (t, J = 6.5 Hz, 1H), 2.43 (t, J = 6.5, 1H), 2.27–2.19 (m, 1H), 1.18–1.15 (9H, m), 1.07 (d, J = 7.0 Hz, 3H). ^{19}F NMR (376 MHz, $CDCl_3$): δ 46.0 (dd, J = 297.0, 54.5 Hz), 45.7 (dd, J = 297.0, 54.5 Hz), 44.9 (dd, J = 297.0, 54.5 Hz), 44.7 (dd, J = 297.0, 54.5 Hz). ^{31}P NMR (162 MHz, $CDCl_3$): δ 149.1, 148.6. HRMS (ESI-TOF): calcd for $C_{40}H_{47}F_2N_4NaO_8P$ $[M + Na]^+$ 803.2997, found 803.2998.

4.3. Synthesis of ***ON1-4***

dU^{CH2F} and dU^{CHF2} phosphoramidites, dA^{Pac}-, $dG^{iPr-Pac}$-, dC^{Ac}-, and dT-phosphoramidites (Glen Research) were used. Syntheses of ONs were performed on a 0.2 μmol scale according to a standard phosphoramidite protocol (coupling reagent = ETT activator, coupling time = 25 s, and phosphoramidite concentration = 0.1 M in MeCN) in DMTr-ON mode.

4.4. Post-synthetic modification of ***ON1*** to ***ON5*** (entry 1 in Table 1)

CPG-attached **ON1** was treated with 50 mM K_2CO_3 in MeOH (1 mL) at room temperature for 4 h. Subsequently, the mixture was diluted with 0.1 M TEAA buffer (pH 7.0, 0.5 mL). After removal of volatile components *in vacuo*, the crude ON was purified on a Sep-Pak® Plus C18 cartridge (Waters) followed by RP-HPLC (Waters XBridge™ Prep Shield RP18 5 μm, 10 × 50 mm) using a linear gradient system [5–15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min; flow rate = 2 mL/min] to afford **ON5** in 33% yield. Deconvoluted ESI-TOF-MS data [M] for **ON5**: found 3010.50 (calcd 3010.01).

4.5. Post-synthetic modification of ***ON1*** to ***ON6*** (entry 3 in Table 1)

CPG-attached **ON1** was treated with 28% NH_3 aqueous solution (1 mL) at room temperature for 2 h. After removal of volatile components *in vacuo*, the crude ON was purified on a Sep-Pak® Plus C18 cartridge (Waters). After removal of volatile components *in vacuo*, the detritylated ON was dissolved in 300 μL of ddH_2O, 10 μL of which was further purified with RP-HPLC (Waters XBridge™ Shield RP18 2.5 μm, 4.6 × 50 mm) using a linear gradient system [5–15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min; flow rate = 1 mL/min] to afford **ON6** in 29% yield along with **ON7** and **ON8** in 9% yield as an inseparable mixture. Deconvoluted ESI-TOF-MS data [M] for **ON6**–**8**: **ON6**, found 2995.50 (calcd 2995.00); **ON7**, found 2996.10 (calcd 2995.98); **ON8**, found 3048.70 (calcd 3048.06).

4.6. Post-synthetic modification of ***ON1*** to ***ON6*** with pre-diethylamine treatment (entry 4 in Table 1)

CPG-attached **ON1** was treated with 20% Et_2NH in MeCN (1 mL) at room temperature for 1 h. The CPG was washed with MeCN (1 mL × 3 times), and then treated with 28% NH_3 aqueous solution (1 mL) at room temperature for 2 h. After removal of volatile components *in vacuo*, the crude ON was purified on a Sep-Pak® Plus C18 cartridge (Waters). After removal of volatile components *in vacuo*, the detritylated ON was dissolved in 300 μL of ddH_2O, 10 μL of which was further purified with RP-HPLC (Waters XBridge™ Shield RP18 2.5 μm, 4.6 × 50 mm) using a linear gradient system [5–15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min; flow rate = 1 mL/min] to afford **ON6** in 37% yield along with **ON7** and **ON9** in 5% and 6% yield, respectively. Deconvoluted ESI-TOF-MS data [M] for **ON9**: found 3051.50 (calcd 3051.11).

4.7. Post-synthetic modification of ***ON3*** to ***ON10*** (entry 1 in Table 2)

CPG-attached **ON3** was treated with 50 mM K_2CO_3 in MeOH (1 mL) at room temperature for 24 h. Subsequently, the mixture was diluted with 0.1 M TEAA buffer (pH 7.0, 0.5 mL). After removal of volatile components *in vacuo*, the crude ON was purified on a Sep-Pak® Plus C18 cartridge (Waters) followed by RP-HPLC (Waters XBridge™ Prep Shield RP18 5 μm, 10 × 50 mm) using a linear gradient system [5–15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min; flow rate = 2 mL/min] to afford **ON10** in 37% yield. Deconvoluted ESI-TOF-MS data [M] for **ON10**: found 3698.10 (calcd 3697.45).

4.8. Post-synthetic modification of ***ON3*** to ***ON11*** (entry 2 in Table 2)

CPG-attached **ON3** was treated with 100 mM NaOH aqueous solution (1 mL) at room temperature for 24 h. Subsequently, the mixture was diluted with 0.1 M TEAA buffer (pH 7.0, 1 mL). After removal of volatile components *in vacuo*, the crude ON was purified on a Sep-Pak® Plus C18 cartridge (Waters) followed by RP-HPLC (Waters XBridge™ Prep Shield RP18 5 μm, 10 × 50 mm) using a linear gradient system [5–15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min; flow rate = 2 mL/min] to afford **ON11** in 19% yield. Deconvoluted ESI-TOF-MS data [M] for **ON11**: found 3683.90 (calcd 3683.42).

4.9. Post-synthetic modification of ***ON3*** to ***ON12*** (entry 3 in Table 2)

CPG-attached **ON3** was treated with 40% $MeNH_2$ aqueous solution (1 mL) at room temperature for 2 h. After removal of volatile components *in vacuo*, the crude ON was purified on a Sep-Pak® Plus C18 cartridge (Waters) followed by RP-HPLC (Waters XBridge™ Prep Shield RP18 5 μm, 10 × 50 mm) using a linear gradient system [5–15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min; flow rate = 2 mL/min] to afford **ON12** in 39% yield. Deconvoluted ESI-TOF-MS data [M] for **ON12**: found 3696.40 (calcd 3696.46).

4.10. Post-synthetic modification of ***ON3*** to ***ON13*** (entry 4 in Table 2)

CPG-attached **ON3** was treated with 20% Et_2NH in MeCN (1 mL)

at 60 °C for 2 h. After cooling to room temperature, the CPG was washed with MeCN (1 mL × 3 times), and then treated with 28% NH_3 aqueous solution (1 mL) at room temperature for 24 h. After removal of volatile components *in vacuo*, the crude ON was purified on a Sep-Pak® Plus C18 cartridge (Waters) followed by RP-HPLC (Waters XBridge™ Prep Shield RP18 5 μm, 10 × 50 mm) using a linear gradient system [5–15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min; flow rate = 2 mL/min] to afford **ON13** in 41% yield. Deconvoluted ESI-TOF-MS data [M] for **ON13**: found 3738.80 (calcd 3738.55).

4.11. Post-synthetic modification of *ON2* to *ON14* (condition A in Scheme 4)

According to the procedure described for synthesis of **ON5**, **ON14** and **ON15** were obtained from CPG-attached **ON2** in 41% and 8% yields, respectively. Deconvoluted ESI-TOF-MS data [M] for **ON14** and **ON15**: **ON14**, found 3016.40 (calcd 3015.97); **ON15**, found 2994.40 (calcd 2993.97).

4.12. Post-synthetic modification of *ON4* to *ON16*

According to the procedure described for synthesis of **ON5**, **ON16** was obtained from CPG-attached **ON4** in 16% yield. Deconvoluted ESI-TOF-MS data [M] for **ON16**: found 3703.90 (calcd 3703.40).

4.13. Post-synthetic modification of *ON16* to *ON17* (entry 2 in Table 3)

ON16 (1 nmol) was treated with 50 mM K_2CO_3 in MeOH (50 μL) at 60 °C for 24 h. After cooling to room temperature, the mixture was diluted with 0.1 M TEAA buffer (pH 7.0, 50 μL). After removal of volatile components *in vacuo*, the crude ON was purified by RP-HPLC (Waters XBridge™ Shield RP18 2.5 μm, 4.6 × 50 mm) using a linear gradient system [5–15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min, flow rate = 1 mL/min] to afford **ON17** in 70% yield. Deconvoluted ESI-TOF-MS data [M] for **ON17**: found 3681.80 (calcd 3681.41).

4.14. Post-synthetic modification of *ON16* to *ON18* (entry 3 in Table 3)

ON16 (1 nmol) was treated with 0.1 M $NH_2OH{\cdot}HCl$ in 0.1 M phosphate buffer (pH 8.0, 50 μL) at room temperature for 24 h. The crude ON was diluted with 0.1 M TEAA buffer (pH 7.0, 50 μL) and purified by RP-HPLC (Waters XBridge™ Shield RP18 2.5 μm, 4.6 × 50 mm) using a linear gradient system [5–15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min, flow rate = 1 mL/min] to afford **ON18** in 27% yield. Deconvoluted ESI-TOF-MS data [M] for **ON18**: found 3697.10 (calcd 3696.42).

4.15. Post-synthetic modification of *ON16* to *ON19* (entry 4 in Table 3)

ON16 (1 nmol) was treated with 0.1 M NH_2NH_2 aqueous solution (50 μL) at 60 °C for 6 h. After cooling to room temperature, the crude ON was diluted with 0.1 M TEAA buffer (pH 7.0, 50 μL) and purified by RP-HPLC (Waters XBridge™ Shield RP18 2.5 μm, 4.6 × 50 mm) using a linear gradient system [5–15% MeCN in 0.1 M TEAA buffer (pH 7.0) for 30 min, flow rate = 1 mL/min] to afford **ON19** in 29% yield. Deconvoluted ESI-TOF-MS data [M] for **ON19**: found 3695.80 (calcd 3695.44).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was partially supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI Grants (JP17K15431).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tet.2020.131769.

References

[1] I. Luyten, P. Herdewijn, Eur. J. Med. Chem. 33 (1998) 515–576.
[2] (a) M.E. Østergaard, P. Kumar, J. Nichols, A. Watt, P.K. Sharma, P. Nielsen, P.P. Seth, Nucleic Acid Therapeut. 25 (2015) 266–274;
(b) T. Moriguchi, H. Sakai, H. Suzuki, K. Shinozuka, Chem. Pharm. Bull. 56 (2008) 1259–1263.
[3] (a) Y. Hagiwara, T. Hasegawa, A. Shoji, M. Kuwahara, H. Ozaki, H. Sawai, Bioorg. Med. Chem. 16 (2008) 7013–7020;
(b) T. Obayashi, M.M. Masud, A.N. Ozaki, H. Ozaki, M. Kuwahara, H. Sawai, Bioorg. Med. Chem. Lett. 12 (2002) 1167–1170.
[4] W.B. Wan, P.P. Seth, J. Med. Chem. 59 (2016) 9645–9667.
[5] Selected examples: (a) F. Allabush, P.M. Mendes, J.H.R. Tucker, RSC Adv. 9 (2019) 31511–31516;
(b) I.N. Michaelides, N. Tago, B. Viverge, T. Carell, Chem. Eur. J. 23 (2017) 15894–15898;
(c) T. Kanamori, A. Takamura, N. Tago, Y. Masaki, A. Okubo, M. Sekine, K. Seio, Org. Biomol. Chem. 15 (2017) 1190–1197.
[6] Recent reviews: (a)A.R. Kapdi, D. Maiti, Y.S. Sanghvi (Eds.), Palladium-Catalyzed Modification of Nucleosides, Nucleotides and Oligonucleotides, Elsevier, 2018;
(b) E. Defrancq, S. Messaoudi, Chembiochem 18 (2017) 426–431;
(c) K.H. Shaughnessy, Molecules 20 (2015) 9419–9454;
(d) M. Merkel, K. Peewasan, S. Arndt, D. Ploschik, H.-A. Wangenknecht, Chembiochem 16 (2015) 1541–1553.
[7] [7] Selected examples: (a) M. Ejlersen, C. Lou, Y.S. Sanghvi, Y. Tor, J. Wengel, Chem. Commun. 54 (2018) 8003–8006;
(b) M.B. Walunj, A.A. Tanpure, S.G. Srivatsan, Nucleic Acids Res. 46 (2018) e65;
(c) L. Lercher, J.F. McGouran, B.M. Kessler, C.J. Schofield, B.G. Davis, Angew. Chem. Int. Ed. 52 (2013) 10553–10558.
[8] Selected examples: (a) O. Romainczyk, B. Endeward, T.F. Prisner, J.W. Engels, Mol. Biosyst. 7 (2011) 1050–1052;
(b) S.I. Khan, M.W. Grinstaff, J. Am. Chem. Soc. 121 (1999) 4704–4705.
[9] (a) A. Krause, A. Hertl, F. Muttach, A. Jäschke, Chem. Eur. J. 20 (2014) 16613–16619;
(b) L. Wicke, J.W. Engels, Bioconjugate Chem. 23 (2012) 627–642.
[10] N. Probst, R. Lartia, O. Théry, M. Alami, E. Defrancq, S. Messaoudi, Chem. Eur. J. 24 (2018) 1795–1800.
[11] Selected examples: (a) C. Wuebben, S. Blume, D. Abdullin, D. Brajtenbach, F. Haege, S. Kath-Schorr, O. Schiemann, Molecules 24 (2019) 4482;
(b) M. Kerzhner, D. Abdullin, J. Więcek, H. Matsuoka, G. Hagelueken, O. Schiemann, M. Famulok, Chem. Eur. J. 22 (2016) 12113–12121;
(c) Á. Eördögh, J. Steinmeyer, K. Peewasan, U. Schepers, H.A. Wagenknecht, P. Kele, Bioconjugate Chem. 27 (2016) 457–464;
(d) C. Dai, L. Wang, J. Sheng, H. Peng, A.B. Draganov, Z. Huang, B. Wang, Chem. Commun. 47 (2011) 3598–3600.
[12] Selected examples: (a) A. Naik, J. Alzeer, T. Triemer, A. Bujalska, N.W. Luedtke, Angew. Chem. Int. Ed. 56 (2017) 10850–10853;
(b) H. Bußkamp, E. Batroff, A. Niederwieser, O.S. Abdel-Rahman, R.F. Winter, V. Wittmann, A. Marx, Chem. Commun. 50 (2014) 10827–10829;
(c) J. Schoch, M. Wiessler, A. Jäschke, J. Am. Chem. Soc. 132 (2010) 8846–8847;
(d) K. Gutsmiedl, D. Fazio, T. Carell, Chem. Eur. J. 16 (2010) 6877–6883.
[13] K. Bartosik, E. Sochacka, G. Leszczynska, Org. Biomol. Chem. 15 (2017) 2097–2103.
[14] Y. Ito, M. Matsuo, K. Yamamoto, W. Yamashita, T. Osawa, Y. Hari, Tetrahedron 74 (2018) 6854–6860.
[15] (a) A.M. Doepner, E.O. Aboagye, A.G.M. Barrett, Tetrahedron Lett. 56 (2015) 3293–3297;
(b) J. Matulic-Adamic, K. Takahashi, T.-C. Chou, H. Gadler, R.W. Price, A.R.V. Reddy, T.I. Kalman, K.A. Watanabe, J. Med. Chem. 31 (1988) 1642–1647;
(c) J. Matulic-Adamic, K.A. Watanabe, J. Chem. Soc., Chem. Commun. (1985)

1535–1536.
[16] M.P. Mertes, S.E. Saheb, D. Miller, J. Med. Chem. 9 (1966) 876–881.
[17] (a) A. Tran, S. Zheng, D.S. White, A.M. Curry, Y. Cen, Chem. Sci. 11 (2020) 11818–11826, https://doi.org/10.1039/D0SC04161K, in press;
(b) M. Janoušková, Z. Vaníková, F. Nici, S. Boháčová, D. Vítovská, H. Šanderová, M. Hocek, L. Krásný, Chem. Commun. 53 (2017) 13253–13255;
(c) F. Kawasaki, P. Murat, Z. Li, T. Santner, S. Balasubramanian, Chem. Commun. 53 (2017) 1389–1392;
(d) T. Pfaffeneder, F. Spada, M. Wagner, C. Brandmayr, S. K Laube, D. Eisen, M. Truss, J. Steinbacher, B. Hackner, O. Kotljarova, D. Schuermann, S. Michalakis, O. Kosmatchev, S. Schiesser, B. Steigenberger, N. Raddaoui, G. Kashiwazaki, U. Müller, C.G. Spruijt, M. Vermeulen, H. Leonhardt, P. Schär, M. Müller, T. Carell, Nat. Chem. Biol. 10 (2014) 574–581.
[18] Z. Vaníková, M. Janoušková, M. Kambová, L. Krásný, M. Hocek, Chem. Sci. 10 (2019) 3937–3942.
[19] J. Matulic-Adamic, K.A. Watanabe, Chem. Pharm. Bull. 36 (1988) 1554–1557.
[20] C. Lou, S.V. Samuelsen, N.J. Christensen, B. Vester, J. Wengel, Bioconjugate Chem. 28 (2017) 1214–1220.
[21] V.W. Armstrong, J.K. Dattagupta, F. Eckstein, W. Saenger, Nucleic Acids Res. 3 (1976) 1791–1810.
[22] (a) A. Ono, T. Okamoto, M. Inada, H. Nara, A. Matsuda, Chem. Pharm. Bull. 42 (1994) 2231–2237;
(b) A. Matsuda, M. Inada, H. Nara, E. Ohtsuka, A. Ono, Bioorg. Med. Chem. Lett. 3 (1993) 2751–2754.
[23] Selected examples: (a) E.A. Belousova, I.A. Vasil'eva, N.A. Moor, T.S. Zatsepin, T.S. Oretskaya, O.I. Lavrik, PloS One 8 (2013), e68576;
(b) M. Tsunoda, T. Sakaue, S. Naito, T. Sunami, N. Abe, Y. Ueno, A. Matsuda, A. Takénaka, J. Nucleic Acids 2010 (2010) 1–10;
(c) H. Kasai, A. Iida, Z. Yamaizumi, S. Nishimura, H. Tanooka, Mutat. Res. 243 (1990) 249–253.
[24] H. Sugiyama, S. Matsuda, K. Kino, Q.-M. Zhang, S. Yonei, I. Saito, Tetrahedron Lett. 37 (1996) 9067–9070.