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Synthesis of Nucleoside Derivatives of *N*-Acetyl-7-nitroindoline, Their Incorporation into the DNA Oligomer, and Evaluation of Their Photoreactivity in the DNA/RNA Duplex

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N-Acetyl-7-nitroindoline has a characteristic reaction in that its acetyl group is photo-activated to acetylate amines to form amides. In this study, the *N*-acetyl-7-nitroindoline part was connected to the 2'-deoxyribose part at the 3- or 5-position or to a glycerol unit at the 3-position through an ethylene linker (1, 2, and 3, respectively). They were incorporated into the oligodeoxynucleotides, and their photo-reactivities toward the complementary RNA were evaluated. The acetyl group of 1 was photo-activated to form the deacetylated nitroso derivative without affecting the RNA strand. The photoreaction with 2 suggested acetylation of the RNA strand. In contrast, compound 3 formed the photo-cross-linked adduct with the RNA. These results have shown the potential application of *N*-acetyl-7-nitroindoline unit in aqueous solutions.

Key words *N*-acetyl-7-nitroindoline; photo-activation; nucleoside analog; oligodeoxynucleotide; photo-acetylation; photo-crosslink

Introduction

The photosolvolysis of the *N*-acetyl-7-nitroindoline derivative (1) was first reported in 1976,¹⁾ and its useful characteristic feature has been applied as a photo-cleavable protecting group of an acetyl group,^{2,3)} and a photo-activatable and clean agent for the ester or amide formation.^{4,5)} Mechanistic studies have established the general mechanism of photoactivation of *N*-acetyl-7-nitroindoline (1) in organic solvents as follows⁶⁾; (i) photo-activation induces the acetyl group transfer to the oxygen atom of the 7-nitro group, resulting in the formation of the active nitronic anhydride (2), (ii) nucleophilic attack to the nitronic anhydride produces a carboxylic acid, an ester or an amide with water, alcohol or amine, respectively, accompanying the formation of the corresponding 7-nitroindoline (3) (Chart 1). When *N*-acetyl-7-nitroindoline derivative (1) is photo-activated in water, the acetic acid is liberated and 7-ni-

troindole (6) is formed instead of 7-nitroindoline. Computational studies⁷⁾ have suggested that the nitronic anhydride (2) becomes unstable by protonation on oxygen atom in water to cause dissociation of AcOH as shown by the arrows in Chart 1. The positively charged nitroso species (4) is produced, which further transforms into 5 and its tautomer nitrosoindole (6). Although it is expected to apply the photo-activatable acetylating property of *N*-acetyl-7-nitroindoline derivative (1) to biological systems, because of the preference of the decomposition of 2 into 4 in water, its application is limited for the use in organic solvents. In a series of our studies for the *in situ* modification of RNA using functionalized oligonucleotides, we were interested in the photo-reactivity of the *N*-acetyl-7-nitroindoline derivative incorporated into the oligonucleotide. The microenvironment of the nucleic acid duplex may provide different circumstances for the reaction rather than a simple aqueous solution. Thus, in this study, the synthesis and photo-reactivity of oligonucleotides containing nucleoside derivatives of an *N*-acetyl-7-nitroindoline skeleton were investigated.

Results and Discussion

In a preliminary study using *N*-acetyl-7-nitroindoline and 3'-*O*-,5'-*O*-bis *tert*-butyldimethylsilyl (TBS)-protected 2'-deoxycytidine in CH₃CN, *N*-acetyl cytidine and 7-nitroindoline

Chart 1. Assumed Mechanism of the Photo-activation of *N*-Acetyl-7-nitroindoline (1)

Fig. 1. Indoline Derivatives Synthesized in This Study

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a) (1) $\text{Pd}(\text{PPh}_3)_4$, Ag_2CO_3 , 1,4-dioxane, 60°C , (2) $\text{Et}_3\text{N}\cdot 3\text{HF}$ in THF, 47%, b) $\text{NaBH}(\text{OAc})_3$, AcOH , CH_3CN , 0°C , 64%, c) (1) DMTrCl , pyridine, 55%, (2) $i\text{Pr}_2\text{N}(\text{P}(\text{Cl})\text{OC}_2\text{H}_4\text{CN})$, DIPEA , CH_2Cl_2 , 0°C , 75%, d) 1) DNA synthesizer, 2) 45 mM K_2CO_3 MeOH, 3) 5% AcOH .

Chart 2. Synthesis of the *N*-Acetyl-7-nitroindoline Nucleoside (7), Its Amidite Derivative (13), and Incorporation into ODN1

were formed by UV irradiation at 365 nm. To investigate the photo-reactivity of the *N*-acetyl-7-nitroindoline derivative in nucleic acids, new nucleoside analogs (7–9) were designed (Fig. 1). Compound 7 connects the indoline unit at the 5 position thus resembling a pyrimidine base shape, and 8 links the indoline at the 3 position giving a similar shape to a purine base. The derivative (9) conjugates the indoline through the noncyclic and flexible spacer.

The syntheses of 7 and its amidite precursor are summarized in Chart 2. 5-Bromo-*N*-acetyl-7-nitroindoline 11 was coupled with glycal 10⁸⁾ by the Mizoroki–Heck reaction using $\text{Pd}(\text{PPh}_3)_4$ and Ag_2CO_3 in 1,4-dioxane at 60°C , followed by the treatment with $\text{Et}_3\text{N}\cdot 3\text{HF}$ in tetrahydrofuran (THF) to produce 12. The reduction of 12 with $\text{NaBH}(\text{OAc})_3$ in CH_3CN in the presence of AcOH at 0°C gave 7, which was transformed into the amidite precursor 13 for use with the DNA synthesizer. The indoline derivative (7) was incorporated into ODN1 in the sequence of 5'-CTTT-7-TTC TCCTTTCT-3'.

In Chart 3, the synthesis of the indoline derivative (8) and its incorporation into the ODN2 are summarized. *N*-Acetyl-3-iodo-7-nitroindole (14) was synthesized from 7-nitroindole by the iodination with *N*-iodosuccinimide, followed by acetylation using sodium hydride (NaH), *N,N*-dimethyl-4-aminopyridine (DMAP) and acetic anhydride in dry THF at 0°C . The Mizoroki–Heck reaction was performed using 14 and 10 to produce the coupled product (15), which was transformed into 16 by reduction under similar conditions described for 7. The reduction of the 2,3-double bond of the indole skeleton of 16 was attempted using a variety of reducing agents such as Pd/C-H_2 , $\text{RhCl}(\text{PPh}_3)_3\text{-H}_2$, NaBH_3CN , triethyl silane, *etc.*, however, none of these reactions produced the desired 8. Considering that the electron-withdrawing nature of the nitro group might influence the 2,3-double bond, we subsequently tried the reduction of the indole skeleton without the nitro group. *N*-Acetyl-3-iodoindole (17) was synthesized from 3-iodoindole⁹⁾ by acetylation using NaH, DMAP and acetic anhydride in dry THF at 0°C . The coupling reaction of 17 with 10 gave 18, which was reduced to 19. The reduction of 19 using Pd/C-H_2 smoothly proceeded in a good yield and the desired indoline skeleton (20) was formed as a 1:1 diastereomixture. The treatment of 20 with *N*-bromosuccinimide (NBS) produced the corresponding 5-bromo derivative (21), which was subjected to nitration with NaNO_3 in trifluoroacetic acid (TFA) to afford the nitrated 22. Debromination of 22 was done with Pd/C-H_2 in the presence of NaHCO_3 to produce the

a) 1) *N*-iodosuccinimide, CH_2Cl_2 , (2) NaH, DMAP, acetic anhydride, THF, 0°C , b) (1) $\text{Pd}(\text{PPh}_3)_4$, Ag_2CO_3 , $\text{P}(\text{C}_6\text{F}_5)_3$, 1,4-dioxane, 60°C , (2) $\text{Et}_3\text{N}\cdot 3\text{HF}$ in THF, 83%, c) $\text{NaBH}(\text{OAc})_3$, AcOH , CH_3CN , 0°C , 89%, d) (1) $\text{Pd}(\text{PPh}_3)_4$, Ag_2CO_3 , $\text{P}(\text{C}_6\text{F}_5)_3$, 1,4-dioxane, 60°C , (2) $\text{Et}_3\text{N}\cdot 3\text{HF}$ in THF, 71%, e) $\text{NaBH}(\text{OAc})_3$, AcOH , CH_3CN , 0°C , 94%, f) Pd/C , H_2 , MeOH, 74%, g) NBS, CH_2Cl_2 , 87%, h) NaNO_3 , TFA, 82%, i) Pd/C , H_2 , NaHCO_3 , MeOH, 54%, j) (1) DMTrCl , pyridine, 55%, (2) $i\text{Pr}_2\text{N}(\text{P}(\text{Cl})\text{OC}_2\text{H}_4\text{CN})$, DIPEA , CH_2Cl_2 , 0°C , 68%, k) 1) DNA synthesizer, 2) 45 mM K_2CO_3 MeOH, 3) 5% AcOH .

Chart 3. Synthesis of the *N*-Acetyl-7-nitroindoline Nucleoside (8), Its Amidite Derivative (23) and Incorporation into ODN2

desired indoline derivative (8). Its amidite precursor 23 was applied to a DNA synthesizer to incorporate 8 into ODN2 in the sequence of 5'-CTTT-8-TTC TCCTTTCT-3'.

The synthesis of the non-cyclic indoline derivative (9) is summarized in Chart 4. 7-Nitroindole was first acetylated at the 3-position using AlCl_3 and ClCOCH_2Br in $\text{ClCH}_2\text{CH}_2\text{Cl}$ to give 24, which was subjected to bromide displacement with (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol in the presence of NaH in dry THF to produce 25. Without purification of 25, treatment with NaBH_3CN in TFA at 0°C accomplished deprotection of the dioxolane protecting group, reduction of the carbonyl group, and also reduction of 2,3-double bond at the same time to afford the desired indoline derivative (26). The 7-nitrogen atom of the indoline part of 26 was acetylated using AlCl_3 and AcCl in $\text{ClCH}_2\text{CH}_2\text{Cl}$ to give 9. The transformation into its amidite precursor 27 and the incorporation into ODN3 in the sequence of 5'-CTTT-9-TTC TCCTTTCT-3' were performed as described above.

Incorporation of the amidite precursor into the ODN was performed using the standard amidite chemistry, and the synthesized ODN was cleaved from the CPG resin in dry MeOH containing 45 mM K_2CO_3 at room temperature for 1 h. The

a) ClCOCH_2Br , AlCl_3 , $\text{ClCH}_2\text{CH}_2\text{Cl}$, 80°C , 65%, b) (S)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol, NaH , THF, 87%, c) NaBH_3CN , TFA, d) AcCl , AlCl_3 , $\text{ClCH}_2\text{CH}_2\text{Cl}$, 80°C , 73%, e) (1) DMTrCl , pyridine, 52%, (2) $i\text{Pr}_2\text{N}(\text{Cl})\text{OC}_2\text{H}_4\text{CN}$, DIPEA , CH_2Cl_2 , 0°C , 83%, f) 1) DNA synthesizer, 2) $45\text{mM K}_2\text{CO}_3$, MeOH , 3) 5% AcOH .

Chart 4. Synthesis of the Non-cyclic Nitroindoline Derivative (**9**), Its Amidite Derivative (**27**) and Incorporation into ODN3

A) After UV irradiation for 7 h.

B) Without UV irradiation

Fig. 2. HPLC Chart of the Photo-reaction Mixture

A) UV-irradiation for 7 h. B) Non-UV irradiation for 7 h. MALDI-TOF/MS suggest followings; peak "a" to be RNA1, peak "b" to be ODN1 with the nitroso derivative (ODN1-b), peak "c" to be ODN1 with the nitro derivative (ODN1-c), and peak "d" to be the non-changed ODN1. UV (365 nm) was irradiated by a 4W UVGL-25 Compact UV lamp to a solution of ODN1 ($5\mu\text{M}$) and RNA1 ($5\mu\text{M}$) in 50mM phosphate buffer containing 100mM NaCl at pH 7.0 and rt. HPLC conditions: Column: SHISEIDO C18, $4.6\times 250\text{mm}$, Column oven: 35°C ; Flow rate: 1.0mL/min ; UV: 254nm. Solvent A: 0.1M TEAA Buffer, Solvent B: CH_3CN , (A) B: 10 to 15% /20min, 15 to 100% /25min, linear gradient. (B) B: 10 to 14% /20min, 40 to 100% /25min, linear gradient.

obtained 4,4'-dimethoxytrityl (DMTr) protected ODN was purified by HPLC using an octadecyl silica (ODS) column, and the DMTr protecting group was removed in 5% aqueous AcOH , then purified again by HPLC. The HPLC charts of the crude DMTr protected ODN and purified ODN are summarized in Figures S1–3. Their UV melting curves and T_m values are summarized in Figure S4.

The photo-reaction was performed using ODN1 incorporating 7 and the complementary RNA1 upon UV irradiation at 365 nm by a 4W UVGL-25 Compact UV lamp, and the reaction mixture was analyzed by HPLC (Fig. 2). After UV irradiation, ODN1 was mostly transformed and only a small por-

A) After UV irradiation for 6 h.

B) MALDI-TOF/MS of the peak a

Fig. 3. A) HPLC Chart of the Photo-reaction Mixture after UV-Irradiation for 6 h; B) The MALDI-TOF/MS Spectra of Peak "a" from the Reaction with RNA1(N = A) after UV Irradiation for 3 and 6 h

Reaction conditions are the same described in the footnote of Fig. 2. HPLC conditions: Column: SHISEIDO C18, $4.6\times 250\text{mm}$, Solvent A: 0.1M TEAA Buffer, Solvent B: CH_3CN , B: 10 to 15% /20min, 15 to 100% /25min, linear gradient; Column oven: 35°C ; Flow rate: 1.0mL/min ; UV: 254nm.

tion was remained unchanged as shown by a peak "d" in Fig. 2A. In contrast, ODN1 is stable and no change was observed without UV irradiation (Fig. 2B). After UV irradiation for 7h, four peaks were observed, which were assigned based on the matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF)/MS; peak "a" was RNA1, peak "b" was ODN1 with the nitroso derivative (ODN1-b), peak "c" was ODN1 with the nitro derivative (ODN1-c), and peak "d" was the non-changed ODN1. The difference in the retention times between HPLC (A) and (B) is due to the different eluent conditions. Only the slightly remaining ODN1 indicated that the photo-activation took place, and preferential formation of ODN1-b with the nitroso species suggested that the active intermediate followed the reaction path as in aqueous media, from **2** to **6** as shown in Chart 1. From the minor production of ODN1-c with the nitro species, nucleophilic attack of the water molecule to the active intermediate **2** might occur to some extent. Acetylation of the amino group of RNA, such that formed in CH_3CN , was not observed in these experiments. Although the duplex between RNA1 and ODN1 is formed based on the UV melting temperature (Fig. S4), the activated nitronic anhydride (**2**) might not be accessible to any nucleophilic amino or hydroxyl group of RNA.

ODN2 incorporating the indoline derivative (**8**) showed a

different photo-reactivity as shown in Fig. 3. In any combination with RNA1 (N = A, G, U, C), no ODN2 remained, but peaks "b" and "c" appeared, which correspond to the ODN2 with the nitroso species and that with the nitro species, respectively, based on their MALDI-TOF/MS. In comparison with ODN1, the increase in the nitro species suggests a more nucleophilic attack to the activated nitrosoindole anhydride (**2**). Interestingly, the intensity of the larger MS peaks ($m/z = 5326.74$ and 5364.81) of the isolated peak "a" became higher with the increasing reaction time compared to the non-changed RNA1 (N = A, $m/z = 5288.21$) as illustrated in Fig. 3B. The observed m/z values of 5326.74 and 5364.81 agree with the calculated values for the mono-acetylated RNA1 ($m/z = 5323.86$) and di-acetylated RNA1 ($m/z = 5365.87$), respectively. Peak "a" of the reaction with the other RNA1 substrate with different N (N = G, U, C) showed a similar tendency in that the intensity of the higher MS value increased with the increased reaction time (Table S1). The non-selective manner for the complementary N in RNA1 suggests that each nucleobase in RNA1 might be acetylated regardless of the position. Furthermore, the formation of the di-acetylated RNA1 implies that acetylation may take place not only in the hybridized duplex but also in an inter-complex fashion. Although further investigation is necessary for the nature of the modification, the increased formation of the nitroindoline species (peak "c") and the acetylation products (higher MS values of peak "a") suggest that the nucleophilic reaction of **2** to **3** is enhanced to some extent with the 3-connected indoline derivative (**8**).

We subsequently investigated the photo-reaction of ODN3 containing the non-cyclic indoline derivative (**9**) after duplex formation with RNA1, and the peaks of the reaction mixture were isolated and analyzed by HPLC (Fig. 4). Peak "a" indicated the non-changed RNA1, and the MS data indicative of acetylation were not observed. Peaks **d** and **e** were shown to be the nitrosoindole and nitroindoline species, respectively (Table S1). It is noteworthy that the MS data of peaks "b" (9997.31) and "c" ($m/z = 10004.0$) are close to the values calculated for the interstrand cross-linked products between RNA1 and ODN3. The reaction mixtures and isolated peaks **b** and **c** gave slow moving bands on the denatured polyacrylamide gel electrophoresis (PAGE) also strongly support the interstrand cross-link formation (Fig. 4B).

The ODNs collected from peaks "d" and "e" were UV-irradiated again in the presence of RNA1 (N = C), but no cross-linked products were formed (Fig. S5), suggesting that the photo-cross link was formed neither with the nitrosoindole species nor nitroindoline species. Based on the MS values, peak "b" ($m/z = 10004.0$) is the adduct between RNA1 (N = C) and ODN3 with the nitrosoindole (or nitrosoindoline) species, and that of peak **c** (9997.31) is the compound formed by further removal of NH_2 or OH group from the above adduct. Regarding the general mechanism for the photoactivation of the *N*-acetyl-7-nitroindoline derivative (**1**), there is no suggestion for the covalent formation between the nucleophile and indoline skeleton (Chart 1). To rationalize the interstrand cross-link, a nucleophilic attack was considered to take place as shown by the arrows marked with "b" in **28** (Chart 5). From the scan calculation performed by changing the bond length between 5-C and the NH_3 nucleophile using DFT, the addition of NH_3 to **28** was estimated to proceed in an exothermic manner without passing through a transition state to

A) After UV irradiation for 6 h.

B) The denatured PAGE analysis of the isolated peaks **a**, **b** and **c**.

Fig. 4. A) HPLC Chart of the Photo-reaction Mixture after UV-Irradiation for 6h; B) The Denatured PAGE Analysis of the Reaction Mixture and the Isolated Peaks **a**, **b** and **c** of the Reaction with RNA1(N = C)

Reaction conditions are the same as described in the footnote of Fig. 2. HPLC conditions: Column: SHISEIDO C18, 4.6×250 mm, Solvent A: 0.1M TEAA Buffer, Solvent B: CH_3CN , B: 10 to 18% /20min, 18 to 100% /25min, linear gradient; Column oven: 35°C ; Flow rate: 1.0 mL/min; UV: 254 nm. Electrophoresis was performed at rt with 20% denatured polyacrylamide gel. Obtained gels were stained with SYBR Gold, which were visualized by a fluorescence image analyzer (LAS-4000).

Chart 5. A Hypothesized Nucleophilic Attack to the Positively Charged Nitrosoindoline Species (**28**)

form **30** with $\Delta G^0 = -0.85 \text{ kcal/mol}$ (Fig. S6). As the energy difference between **28** and **30** is very small, the NH_3 group of **30** should easily eliminate and dissociate from **28**. When **30** aromatizes to **31** by the proton transfer, the C5- NH_3 bond becomes non-cleavable. In contrast to the predominant formation of **29** by deprotonation in water (arrows marked with "a" in Chart 5), the formation of adduct **31** suggests that a nucleophile is placed in close proximity to the charged species **28** in duplex between ODN3 and RNA1. Although the structure determination of the cross-linked adducts is necessary for

further discussion, the possibility of a photo-activated bond formation to the charged species may expand the utility of the *N*-acetyl-7-nitroindoline.

Conclusion

N-Acetyl-7-nitroindoline is known to be activated by UV-irradiation to form nitronic anhydride (**2**), which is used for the acid liberation and the formation of ester and amide bonds. A drawback of *N*-acetyl-7-nitroindoline is that the nitronic anhydride (**2**) decomposes in water and it is difficult to be used for the bond forming reaction. In this study, we synthesized three nucleoside derivatives connecting *N*-acetyl-7-nitroindoline (**7**, **8** and **9**) and incorporated them into the oligodeoxynucleotides. By UV-irradiation to the duplex formed between ODN1 incorporating **7** and complementary RNA1, the *N*-acetyl-7-nitroindoline moiety was activated, and the nitroso species was formed. Thus, the reaction environment of the *N*-acetyl-7-nitroindoline moiety of **7** in the duplex is similar with the bulk water phase. In contrast, ODN2 incorporating **8** produced the acetylated products of the target RNA1 in a non-selective manner against the target nucleobase. Although a further detailed study is necessary to clarify the acetylated structures, the activated nitronic anhydride may be applicable to acetylation in a specialized environment such as in the duplex. An interesting property of the *N*-acetyl-7-nitroindoline was observed when it is linked to the non-cyclic derivative (**9**). ODN3 incorporating **9** produced an interstrand cross-linking with the complementary RNA1 in a non-selective manner for the target nucleobase. Although detailed structure determination is necessary, the interstrand crosslink formation suggests that a charged nitroso species **4** may accept an intermolecular nucleophilic attack in a specialized environment such as in the duplex. Thus, these results have shown the potential application of *N*-acetyl-7-nitroindoline unit in aqueous solutions.

Experimental

General ^1H , ^{13}C , and ^{31}P -NMR spectra were recorded by (^1H -NMR, 400 or 500 MHz; ^{13}C -NMR, 125 MHz; ^{31}P -NMR, 161 or 202 MHz) spectrometers. The 2D-NMRs (H-correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond connectivity (HMBC)) were used for the assignment of some of the intermediates. The chemical shifts (δ) are reported in parts per million (ppm) relative to the residual solvent signal for ^1H , and the residual solvent signal for ^{13}C . The ^{31}P spectra were recorded using an external reference 10% D_2O phosphate (0.00 ppm). The chemical shifts are described as s (singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublet of doublets), t (triplet), dt (doublet of triplets), q (quartet), m (multiplet), and bs (broad signal). The coupling constants (J) are reported in hertz. The electrospray ionization (ESI)-MS were acquired by a time-of-flight mass spectrometer using two of physalaemin, neurotensin, angiotensin I, bradykinin, and nicotinic acid as the internal standards (Applied Biosystems Mariner Biospectrometry Workstation). MALDI-TOF/MS spectra were recorded by BRUKER DALTONICS microflex-KS Linear using an aqueous solution of 3-hydroxypicolinic acid and diammonium hydrogencitrate. The IR spectra were recorded by PerkinElmer, Inc. SpectrumOne FT-IR spectrometer. The UV-VIS spectra and UV-melting curves were obtained by BECKMAN COULTER DU 800. The column silica

gel chromatographic separations were carried out by gradient elution with the suitable combination of solvents and silica gel (Kanto Silicagel 60N or Fuji gel FL-60D). The reagents and anhydrous solvents were purchased from commercial sources and used without further purification unless otherwise stated. Flash-chromatography was performed using a YAMAZEN AI0580S with prepacked columns (BiotageZIPTM, 10, 30, 45, 120 g). The HPLC was performed using a JASCO LC-2000PLUS equipped with a column oven using SHISEIDO CAPCELL PAK C18 MG (4.6×250 nm) for analysis and Nacalai Tesque COSMOSIL 5C₁₈-AR-II for purification. The oligonucleotides were synthesized by an Applied Biosystems 394 DNA/RNA Synthesizer or NTS H-Series DNA/RNA Synthesizer. The RNA substrates were commercially purchased and used without further purification. The photoreactions were performed using a Funakoshi 4W handy UV lamp UVGL-25 equipped with a filter for 365 nm.

1' β -(*N*-Acetyl-7-nitroindoline-5-yl)-1',2',3'-trideoxy-3'-oxo-D-ribofuranose (12**)** Under an Ar atmosphere, 5-bromo-*N*-acetyl-7-nitroindoline (30 mg, 0.105 mmol) and glycol (48.4 mg, 0.210 mmol) were dissolved in 1,4-dioxane (0.8 mL). After the solution was degassed, Ag_2CO_3 (57.9 mg, 0.210 mmol) and $\text{Pd}(\text{PPh}_3)_4$ were added, and the reaction mixture was stirred at 60 °C for 20 h. The precipitates were filtered off through a Celite pad and the filtrate was evaporated. The residue was dissolved in dry THF (0.4 mL) under an Ar atmosphere. $\text{Et}_3\text{N} \cdot 3\text{HF}$ (82 μL , 0.504 mmol) was added at 0 °C, and the reaction was stirred at room temperature for 30 min. The reaction mixture was evaporated and the residue was purified by flash chromatography on silica gel (hexane/EtOAc = 1:2 to 1:19) to obtain **12** (15.8 mg, 49.4 μmol , 47%) as a yellow oil. ^1H -NMR (500 MHz, CDCl_3) δ (ppm): 7.71 (1H, s), 7.55 (1H, s), 5.23 (1H, dd, $J = 10.9, 6.0$ Hz), 4.27 (2H, t, $J = 8.0$ Hz), 4.07 (1H, t, $J = 3.4$ Hz), 3.98 (2H, d, $J = 3.4$ Hz), 2.91 (1H, dd, $J = 17.9, 6.0$ Hz), 2.46 (1H, dd, $J = 17.9, 10.9$ Hz), 2.28 (3H, s); ^{13}C -NMR (125 MHz, CD_3OD) δ (ppm): 212.6, 167.1, 131.7, 126.0, 125.0, 124.7, 123.9, 121.2, 121.1, 82.1, 71.1, 61.4, 60.4, 43.3, 23.4, 14.20; IR (neat): 1757, 1681, 1536 cm^{-1} ; ESI-HRMS (m/z) Calcd. for $\text{C}_{15}\text{H}_{16}\text{N}_2\text{O}_6\text{Na}$ [$\text{M} + \text{Na}$]⁺: 343.0901, Found: 343.0929.

1' β -(*N*-Acetyl-7-nitroindoline-5-yl)-1',2'-dideoxy-D-ribofuranose (7**)** Under an Ar atmosphere, **12** (21.1 mg, 65.9 μmol) was dissolved in CH_3CN (590 μL) at 0 °C. Then, AcOH (66 μL) and $\text{NaBH}(\text{OAc})_3$ (21.0 mg, 98.9 μmol) were added, and the reaction was stirred at 0 °C for 30 min. The reaction mixture was evaporated and the residue was dissolved in CH_2Cl_2 . The solution was washed with aqueous saturated NaHCO_3 , dried with Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography on silica gel ($\text{CHCl}_3/\text{MeOH} = 19:1$ to $16:1$) to obtain **7** (13.5 mg, 42.2 μmol , 64%) as a brown foam. ^1H -NMR (500 MHz, CD_3OD) δ (ppm): 7.67 (1H, d, $J = 1.0$ Hz), 7.60 (1H, d, $J = 1.0$ Hz), 5.15 (1H, dd, $J = 10.3, 5.3$ Hz), 4.33 (1H, ddd, $J = 5.9, 2.3, 2.0$ Hz), 4.29 (2H, t, $J = 8.0$ Hz), 3.95 (1H, dt, $J = 5.0, 2.3$ Hz), 3.68 (1H, d, $J = 5.0$ Hz), 3.23 (2H, t, $J = 8.0$ Hz) 2.26–2.22 (4H, m), 1.92 (1H, ddd, $J = 13.1, 10.3, 5.9$ Hz); ^{13}C -NMR (125 MHz, CD_3OD) δ (ppm): 171.2, 142.1, 141.4, 139.2, 134.5, 127.6, 120.9, 89.4, 80.3, 74.3, 63.9, 51.7, 45.0, 29.9, 23.1; IR (neat): 3381, 1655, 1535 cm^{-1} ; ESI-HRMS (m/z) Calcd. for $\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}_6\text{Na}$ [$\text{M} + \text{Na}$]⁺: 345.1057, Found: 345.1054.

1' β -(*N*-Acetyl-7-nitroindoline-5-yl)-1',2'-dideoxy-5'-O-

(4,4'-dimethoxytriphenyl-methyl)-D-ribofuranose-3-[(2-cyanoethyl)(*N,N*-diisopropyl)]phosphoramidite (13**)** Under an Ar atmosphere, **7** (50 mg, 0.155 mmol) was dried by azeotrope with CH₃CN and pyridine. The residue was dissolved in pyridine (1.2 mL). Then, DMTrCl (131.5 mg, 0.388 mmol) was added at 0 °C, and the reaction was stirred at room temperature for 1 h. The reaction was quenched with aqueous saturated NaHCO₃ and extracted with AcOEt. The organic layer was washed with brine, dried with Na₂SO₄ and evaporated to dryness. The residue was purified by flash chromatography on silica gel (hexane/EtOAc = 2:3 to 1:7) to obtain the 5'-*O*-(4,4'-dimethoxytriphenylmethyl) derivative of **7** (53.5 mg, 85.3 μmol, 55%) as a pale yellow foam. ¹H-NMR (500 MHz, CD₃OD) δ (ppm): 7.62 (1H, s), 7.50 (1H, s), 7.44–7.42 (2H, m), 7.33–7.31 (4H, m), 7.29–7.26 (2H, m), 7.23–7.20 (1H, m), 6.84–6.82 (4H, m), 5.17 (1H, dd, *J* = 10.0, 5.5 Hz), 4.44–4.43 (1H, m), 4.21 (2H, t, *J* = 7.9 Hz), 4.08–4.07 (1H, m), 3.79 (6H, s), 3.35 (1H, dd, *J* = 9.8, 4.3 Hz), 3.28 (1H, dd, *J* = 9.8, 5.0 Hz), 3.10 (2H, dt, *J* = 7.9, 4.0 Hz), 2.29–2.25 (4H, m), 2.03–1.99 (1H, m); ¹³C-NMR (125 MHz, CD₃OD) δ (ppm): 168.5, 158.7, 144.9, 140.7, 139.6, 136.9, 136.0, 136.0, 133.9, 130.2, 128.3, 128.0, 127.0, 126.4, 120.3, 113.3, 86.7, 86.5, 78.9, 74.7, 60.6, 50.4, 44.1, 29.1, 23.4; IR (neat): 1678, 1608, 1537, 1508 cm⁻¹; ESI-HRMS (*m/z*) Calcd. for C₃₆H₃₆N₂O₈Na [M + Na]⁺: 647.2364, Found: 647.2380.

Under an Ar atmosphere, the above compound (53.3 mg, 85.3 μmol) was dried by azeotrope with CH₃CN and dissolved in CH₂Cl₂ (0.78 mL). Then, *N,N*-diisopropylethylamine (DIPEA) (57 μL, 256 μmol) and 2-cyanoethyl *N,N*-diisopropylchloro-phosphoramidite (57 μL, 256 μmol) were added. After stirring at 0 °C for 1 h, the reaction mixture was quenched with aqueous saturated NaHCO₃ and extracted with CH₂Cl₂. The organic layer was washed with brine, dried with Na₂SO₄ and evaporated to dryness. The residue was purified by flash chromatography on silica gel (hexane/EtOAc = 1:1) to obtain the material, which was crystallized in hexane at -78 °C. The solvents were removed by decantation, and the solid material was dried in a vacuum to give **13** (52.5 mg, 70.0 μmol, 75%) as a pale yellow foam. ¹H-NMR (500 MHz, CD₃OD) δ (ppm): 7.67 (0.5H, d, *J* = 1.0 Hz), 7.64 (0.5H, d, *J* = 1.0 Hz), 7.56 (0.5H, d, *J* = 1.0 Hz), 7.54 (0.5H, d, *J* = 1.0 Hz), 7.45–7.43 (2H, m), 7.34–7.31 (4H, m), 7.29–7.25 (2H, m), 7.23–7.20 (1H, m), 6.83–6.81 (4H, m), 5.16 (1H, dd, *J* = 10.0, 5.5 Hz), 4.54–4.51 (1H, m), 4.25–4.19 (3H, m), 3.88–3.82 (0.5H, m), 3.80 (3H, s), 3.79 (3H, s), 3.78–3.75 (0.5H, m), 3.74–3.65 (1H, m), 3.63–3.55 (2H, m), 3.36–3.24 (2H, m), 3.11–3.08 (2H, m), 2.62 (1H, d, *J* = 6.5 Hz), 2.46–2.33 (2H, m), 2.25 (3H, s), 2.05–1.97 (1H, m), 1.19 (3H, d, *J* = 5.5 Hz), 1.18 (3H, d, *J* = 6.5 Hz), 1.16 (3H, d, *J* = 6.5 Hz), 1.09 (3H, d, *J* = 7.0 Hz); ³¹P-NMR (202 MHz, CDCl₃) δ (ppm): 148.19, 148.05; IR (neat): 1680, 1608, 1536, 1509 cm⁻¹; ESI-HRMS (*m/z*) Calcd. for C₄₅H₅₃N₄O₉PNa [M + Na]⁺: 847.3442, Found: 847.3442.

***N*-Acetyl-3-iodo-7-nitroindole (**14**)** Under an Ar atmosphere, 7-nitroindole (500 mg, 3.1 mmol) was dissolved in CH₂Cl₂ (9.0 mL), and *N*-iodosuccinimide (765 mg, 3.4 mmol) was added. The reaction mixture was stirred for 1 h. The precipitates were filtered off and yellow amorphous solids were obtained. The filtrate was evaporated and the residue was purified by flash chromatography on silica gel (hexane/EtOAc = 99:1) to obtain yellow amorphous solids. The yellow amorphous solids were recrystallized from hexane, EtOAc

and CH₂Cl₂ to give 3-iodo-7-nitroindole (762.6 mg, 2.64 mmol, 85%) as a yellow needle crystal. ¹H-NMR (500 MHz, CDCl₃) δ (ppm): 10.07 (1H, br), 8.24 (1H, d, *J* = 7.9 Hz), 7.83 (1H, dd, *J* = 7.6 Hz), 7.50 (1H, d, *J* = 2.0 Hz), 7.31 (1H, dd, *J* = 7.9, 7.6 Hz); ¹³C-NMR (125 MHz, CDCl₃) δ (ppm): 133.5, 133.2, 131.1, 129.5, 129.4, 120.4, 120.1, 59.1; IR (neat): 3348, 1520, 1494, 1480 cm⁻¹; ESI-HRMS (*m/z*) Calcd. for C₈H₅IN₂O₂Na [M + Na]⁺: 310.9293, Found: 310.9264.

Under an Ar atmosphere, a mixture of 3-iodo-7-nitroindole obtained above (1.0 g, 3.47 mmol) and NaH (60%, 696 mg, 17.4 mmol) in THF (36 mL) was stirred for 3 h. Then, DMAP (42.4 mg, 0.347 mmol) and acetic anhydride (1.65 mL, 17.4 mmol) were added at 0 °C and the reaction mixture was stirred at room temperature for 20 h. The reaction was quenched with aqueous saturated NaHCO₃ and extracted with EtOAc. The organic layer was washed with aqueous saturated NH₄Cl and brine, dried with Na₂SO₄ and evaporated to dryness. The residue was purified by flash chromatography on silica gel (hexane/EtOAc = 19:1 to 6:1) to obtain **14** (815.3 mg, 2.46 mmol, 71%) as a beige amorphous solid. ¹H-NMR (500 MHz, CDCl₃) δ (ppm): 7.85 (1H, dd, *J* = 7.8, 0.9 Hz), 7.70 (1H, dd, *J* = 7.9, 0.9 Hz), 7.68 (1H, s), 7.47 (1H, dd, *J* = 7.9, 7.8 Hz), 2.66 (3H, s); ¹³C-NMR (125 MHz, CDCl₃) δ (ppm): 166.4, 140.0, 135.6, 131.9, 126.6, 125.00, 124.3, 121.7, 66.7, 23.5; IR (neat): 1731, 1532, 1423, 1375 cm⁻¹; ESI-HRMS (*m/z*) Calcd. for C₁₀H₇IN₂O₃Na [M + Na]⁺: 352.9394, Found: 352.9399.

1'β-(*N*-Acetyl-7-nitroindole-3-yl)-1',2',3'-trideoxy-3'-oxo-D-ribofuranose (15**)** Under an Ar atmosphere, **14** (818.6 mg, 2.48 mmol) and Glycal (628.9 mg, 2.73 mmol) were dissolved in 1,4-dioxane (19 mL). After the solution was degassed, Ag₂CO₃ (1.367 g, 4.96 mmol), tris(pentafluorophenyl)phosphine (527.9 mg, 0.992 mmol) and Pd(OAc)₂ (111.3 mg, 0.496 mmol) were added, and the reaction was stirred at 60 °C for 1 h. The precipitates were filtered off through a Celite pad and the filtrate was evaporated. The residue was dissolved in dry THF (9.5 mL) under an Ar atmosphere. Et₃N·3HF (1.94 mL, 11.9 mmol) was added at 0 °C, and the reaction was stirred at room temperature for 30 min. The reaction mixture was evaporated and the residue was purified by flash chromatography on silica gel (hexane/EtOAc = 1:1 to 1:2 to 1:4) to obtain **15** (653.3 mg, 2.09 mmol, 83%, 2 steps) as a pale yellow amorphous solid. ¹H-NMR (500 MHz, CDCl₃) δ (ppm): 8.00 (1H, dd, *J* = 8.0, 1.0 Hz), 7.81 (1H, dd, *J* = 7.9, 0.8 Hz), 7.61 (1H, s), 7.57 (1H, dd, *J* = 8.0, 7.9 Hz), 5.51 (1H, ddd, *J* = 10.9, 5.9, 0.8 Hz), 4.12 (1H, t, *J* = 3.3 Hz), 4.00 (2H, t, *J* = 3.3 Hz), 2.97 (1H, dd, *J* = 17.9, 5.9 Hz), 2.76 (1H, dd, *J* = 17.9, 10.9 Hz), 2.66 (3H, s); ¹³C-NMR (125 MHz, CDCl₃) δ (ppm): 212.8, 167.3, 140.5, 131.9, 126.1, 125.1, 124.9, 124.0, 121.4, 121.2, 82.3, 71.4, 61.5, 43.4, 23.6; IR (neat): 3567, 1758, 1728, 1531, 1432, 1372 cm⁻¹; ESI-HRMS (*m/z*) Calcd. for C₁₅H₁₄N₂O₆Na [M + Na]⁺: 341.0744, Found: 341.0773.

1'β-(*N*-Acetyl-7-nitroindole-3-yl)-1',2'-dideoxy-D-ribofuranose (16**)** Under an Ar atmosphere, **15** (50.0 mg, 0.157 mmol) was dissolved in CH₃CN (1.41 mL) at 0 °C. Then, AcOH (157 μL) and NaBH(OAc)₃ (50.0 mg, 0.236 mmol) were added, and the reaction was stirred at 0 °C for 3.5 h. The reaction mixture was evaporated and the residue was purified by flash chromatography on silica gel (CHCl₃/MeOH = 19:1 to 9:1) to obtain **16** (44.6 mg, 0.139 mmol, 89%) as a yellow foam. ¹H-NMR (500 MHz, CD₃OD) δ (ppm): 8.06 (1H,

dd, $J=8.0$, 1.0 Hz), 7.75 (1H, d, $J=8.0$, 1.0 Hz), 7.43 (1H, t, $J=8.0$ Hz), 5.42 (1H, dd, $J=10.0$, 5.5 Hz), 4.41 (1H, ddd, $J=6.0$, 2.7, 2.3 Hz), 3.98 (1H, ddd, $J=4.8$, 4.4, 2.7 Hz), 3.73 (1H, dd, $J=11.6$, 4.4 Hz), 3.70 (1H, dd, $J=11.6$, 4.8 Hz), 2.66 (3H, s), 2.32 (1H, ddd, $J=13.0$, 5.5, 2.3 Hz); ^{13}C -NMR (125 MHz, CD_3OD) δ (ppm): 169.7, 141.7, 133.9, 126.7, 126.0, 124.5, 124.2, 121.4, 89.2, 74.8, 74.1, 63.7, 42.8, 23.3; IR (neat): 3399, 1725, 1531 cm^{-1} ; ESI-HRMS (m/z) Calcd. for $\text{C}_{15}\text{H}_{16}\text{N}_2\text{O}_6\text{Na}$ [$\text{M} + \text{Na}$] $^+$: 343.0901, Found: 343.0901.

***N*-Acetyl-3-iodoindole (17)** Under an Ar atmosphere, indole (2.0 g, 17.1 mmol) was dissolved in dry DMF (40 mL). Then, KOH (2.4 g, 42.8 mmol) and iodine (4.34 g, 17.1 mmol) were added and the reaction mixture was stirred for 30 min. The reaction mixture was poured ice water (200 mL) containing ammonia (0.5%) and sodium metabisulphite (0.1% aqueous solution). The precipitate was filtered and washed with cold water to give 3-iodoindole (3.52 g, 14.5 mmol, 85%) as pale brown amorphous solid. ^1H -NMR (500 MHz, dimethyl sulfoxide (DMSO)- d_6) δ (ppm): 11.54 (1H, br), 7.55 (1H, d, $J=2.5$ Hz), 7.41 (1H, d, $J=7.8$ Hz), 7.27 (1H, d, $J=7.7$ Hz), 7.16 (1H, ddd, $J=7.5$, 7.4, 0.9 Hz), 7.10 (1H, ddd, $J=7.7$, 7.4, 0.9 Hz); ^{13}C -NMR (125 MHz, DMSO - d_6) δ (ppm): 135.9, 129.7, 129.3, 122.2, 119.9, 119.8, 111.9, 56.0.

Under an Ar atmosphere, a mixture of 3-iodoindole obtained above (3.52 g, 3.47 mmol) and NaH (60%, 1.392 g, 34.8 mmol) in THF (120 mL) was stirred for 45 min. Then, DMAP (247 mg, 1.45 mmol) and acetic anhydride (3.3 mL, 34.8 mmol) were added at 0°C and the reaction mixture was stirred at room temperature for 20 h. The reaction mixture was evaporated and the residue was dissolved in EtOAc. The solution was washed with aqueous saturated NaHCO_3 , aqueous saturated NH_4Cl and brine, dried with Na_2SO_4 and evaporated to obtain **17** (4.05 g, 3.40 mmol, 98%) as a pale yellow amorphous solid. ^1H -NMR (500 MHz, DMSO - d_6) δ (ppm): 8.32 (1H, d, $J=8.0$ Hz), 8.15 (1H, s), 7.41–7.37 (3H, m), 2.65 (3H, s); ^{13}C -NMR (125 MHz, DMSO - d_6) δ (ppm): 167.8, 135.2, 132.2, 129.6, 126.4, 124.5, 121.6, 116.6, 68.1, 24.1; ESI-HRMS (m/z) Calcd. for $\text{C}_{10}\text{H}_8\text{INa}$ [$\text{M} + \text{Na}$] $^+$: 307.9543, Found: 307.9561.

***1* β -(*N*-Acetylindole-3-yl)-1',2',3'-trideoxy-3'-oxo-*D*-ribofuranose (18)** Under an Ar atmosphere, **17** (650 mg, 2.28 mmol) and glycol (579.3 mg, 2.51 mmol) were dissolved in 1,4-dioxane (17.5 mL). After the solution was degassed, Ag_2CO_3 (1.257 g, 4.56 mmol), tris(penta-fluorophenyl)-phosphine (204.7 mg, 0.912 mmol) and $\text{Pd}(\text{OAc})_2$ (102.4 mg, 0.456 mmol) were added, and the reaction was stirred at 60°C for 40 min. The precipitates were filtered off through a Celite pad and the filtrate was evaporated. The residue was dissolved in dry THF (8.8 mL) under an Ar atmosphere. $\text{Et}_3\text{N} \cdot 3\text{HF}$ (1.8 mL, 10.9 mmol) was added at 0°C, and the reaction was stirred at room temperature for 30 min. The reaction mixture was evaporated and the residue was purified by flash chromatography on silica gel (hexane/EtOAc = 1:1 to 1:4) to obtain **18** (441.2 mg, 1.62 mmol, 71%, 2 steps) as a yellow foam. ^1H -NMR (500 MHz, CDCl_3) δ (ppm): 8.45 (1H, d, $J=7.6$ Hz), 7.66 (1H, d, $J=7.5$ Hz), 7.49 (1H, s), 7.40 (1H, dt, $J=7.6$, 0.8 Hz), 7.31 (1H, ddd, $J=7.6$, 7.5, 0.8 Hz), 5.48 (1H, ddd, $J=10.8$, 5.9, 0.6 Hz), 4.10 (1H, t, $J=3.5$ Hz), 3.98 (2H, d, $J=3.5$ Hz), 2.94 (1H, dd, $J=17.9$, 5.9 Hz), 2.79 (1H, dd, $J=17.9$, 10.8 Hz), 2.65 (3H, s); ^{13}C -NMR (125 MHz, CDCl_3) δ (ppm): 213.5, 168.7, 136.5, 128.2, 126.0, 124.1, 122.7,

121.3, 119.7, 117.1, 82.2, 71.9, 61.5, 43.4, 24.2; ESI-HRMS (m/z) Calcd. for $\text{C}_{15}\text{H}_{15}\text{NO}_4\text{Na}$ [$\text{M} + \text{Na}$] $^+$: 296.0893, Found: 296.0876.

***1* β -(*N*-Acetylindole-3-yl)-1',2'-dideoxy-*D*-ribofuranose (19)** Under an Ar atmosphere, **18** (341.2 mg, 1.25 mmol) was dissolved in CH_3CN (11.3 mL) at 0°C. Then, AcOH (1.2 mL) and $\text{NaBH}(\text{OAc})_3$ (398.4 mg, 1.88 mmol) were added, and the reaction was stirred at 0°C for 3 h. The reaction mixture was evaporated and the residue was purified by flash chromatography on silica gel ($\text{CHCl}_3/\text{MeOH}=1:0$ to 9:1) to obtain **19** (324.6 mg, 1.18 mmol, 94%) as a yellow foam. ^1H -NMR (500 MHz, CD_3OD) δ (ppm): 8.37 (1H, d, $J=8.1$ Hz), 7.70 (1H, d, $J=0.5$ Hz), 7.70–7.66 (1H, m), 7.31 (1H, ddd, $J=8.1$, 7.7, 1.1 Hz), 7.26 (1H, ddd, $J=7.7$, 7.6, 1.1 Hz), 5.38 (1H, dd, $J=10.4$, 5.8 Hz), 4.38 (1H, ddd, $J=6.0$, 2.7, 2.1 Hz), 3.96 (1H, dt, $J=4.9$, 2.7 Hz), 3.71 (2H, t, $J=4.9$ Hz), 2.64 (3H, s), 2.29 (1H, ddd, $J=13.0$, 5.8, 2.1 Hz), 2.21 (1H, ddd, $J=13.0$, 10.4, 6.0 Hz); ^{13}C -NMR (125 MHz, CD_3OD) δ (ppm): 171.2, 137.6, 130.2, 126.1, 124.6, 124.4, 123.9, 120.8, 117.5, 89.0, 75.2, 74.2, 63.8, 42.7, 23.9; ESI-HRMS (m/z) Calcd. for $\text{C}_{15}\text{H}_{17}\text{NO}_4\text{Na}$ [$\text{M} + \text{Na}$] $^+$: 298.1050, Found: 298.1010.

***1* β -(*N*-Acetylindoline-3-yl)-1',2'-dideoxy-*D*-ribofuranose (20)** After three vacuum/ H_2 cycles to remove air from the round-bottom flask, a mixture of **19** (30.7 mg, 112 μmol), 10% Pd/C (3.1 mg, 29.1 μmol) in methanol (3.7 mL) was stirred at room temperature for 4 h under ambient pressure of hydrogen (balloon). The reaction mixture was filtered through a Celite pad, and the filtrate was evaporated. The residue was purified by flash chromatography on silica gel ($\text{CHCl}_3/\text{MeOH}=1:0$ to 9:1) to obtain **20** (23.0 mg, 82.9 μmol , 74%, $dr=50:50$) as a pale yellow amorphous solid. ^1H -NMR (500 MHz, CD_3OD) δ (ppm): 8.10 (0.5H, d, $J=8.0$ Hz), 8.08 (0.5H, d, $J=8.0$ Hz), 7.40 (1H, d, $J=7.5$ Hz), 7.31 (1H, d, $J=7.5$ Hz), 7.21–7.18 (1H, m), 7.06–7.02 (1H, m), 4.36 (0.5H, ddd, $J=10.4$, 5.8, 5.6 Hz), 4.25 (0.5H, ddd, $J=10.4$, 5.8, 5.4 Hz), 4.21–4.19 (1H, m), 4.15–4.12 (1H, m), 4.10–4.04 (1H, m), 3.84 (0.5H, dt, $J=4.5$, 2.5 Hz), 3.78 (0.5H, dt, $J=4.9$, 2.8 Hz), 3.71–3.68 (0.5H, m), 3.60–3.51 (0.5H, m), 2.24 (1.5H, s), 2.23 (1.5H, s), 1.95 (0.5H, ddd, $J=13.0$, 5.4, 1.8 Hz), 1.83 (0.5H, ddd, $J=13.0$, 10.4, 6.4 Hz), 1.75 (0.5H, ddd, $J=13.1$, 10.4, 6.4 Hz), 1.60 (0.5H, ddd, $J=13.1$, 5.6, 2.0 Hz); ^{13}C -NMR (125 MHz, CD_3OD) δ (ppm): 171.5, 171.4, 144.4, 144.0, 134.1, 133.4, 129.1, 129.0, 126.4, 126.2, 125.0, 125.0, 117.9, 89.1, 88.7, 82.0, 81.6, 74.1, 73.7, 63.9, 63.9, 52.8, 51.5, 45.8, 44.8, 39.5, 37.9, 24.0; IR (neat): 3373, 1641, 1594, 1483, 1460, 1415 cm^{-1} ; ESI-HRMS (m/z) Calcd. for $\text{C}_{15}\text{H}_{19}\text{NO}_4\text{Na}$ [$\text{M} + \text{Na}$] $^+$: 300.1206, Found: 300.1212.

***1* β -(*N*-Acetyl-5-bromoindoline-3-yl)-1',2'-dideoxy-*D*-ribofuranose (21)** Under an Ar atmosphere, **20** (19.1 mg, 68.9 μmol) was dissolved in dry CH_2Cl_2 (0.56 mL). Then, *N*-boromosuccinimide (13.6 mg, 76.3 μmol) was added and the reaction mixture was stirred for 7 h. The reaction mixture was evaporated and the residue was purified by flash chromatography on silica gel ($\text{CHCl}_3/\text{MeOH}=1:0$ to 19:1 to 9:1) to obtain **21** (21.6 mg, 59.9 μmol , 87%, $dr=50:50$) as a pale-yellow amorphous solid. ^1H -NMR (500 MHz, MeOD) δ (ppm): 8.01 (1H, d, $J=8.5$ Hz), 8.0 (1H, d, $J=8.5$ Hz), 7.53 (0.5H, d, $J=1.0$ Hz), 7.45 (0.5H, d, $J=1.0$ Hz), 7.34–7.31 (1H, m), 4.30 (1H, dt, $J=10.4$, 5.4 Hz), 4.24–4.22 (0.5H, m), 4.20–4.13 (2H, m), 4.02 (1H, dd, $J=11.3$, 5.3 Hz), 3.85 (0.5H, dt, $J=4.5$, 2.5 Hz), 3.78 (0.5H, dt, $J=4.9$, 2.8 Hz), 3.67–3.64 (0.5H, m),

3.63–3.58 (1.5H, m), 3.54 (1H, t, $J=4.9$ Hz), 2.23 (1.5H, s), 2.22 (0.5H, s), 1.93 (0.5H, ddd, $J=12.9, 5.4, 1.6$ Hz), 1.81–1.69 (1.5H, m); ^{13}C -NMR (125 MHz, MeOD) δ (ppm): 171.6, 171.5, 143.8, 143.4, 136.9, 136.2, 131.9, 131.8, 129.7, 129.3, 119.3, 117.2, 117.0, 89.2, 88.7, 81.8, 81.1, 74.0, 73.8, 63.8, 63.8, 52.6, 51.8, 45.2, 45.1, 39.1, 38.5, 24.0; ESI-HRMS (m/z) Calcd. for $\text{C}_{15}\text{H}_{18}\text{BrNO}_4\text{Na}$ [$\text{M} + \text{Na}$] $^+$: 378.0311, 380.0292, Found: 378.0310, 380.0289.

1' β -(*N*-Acetyl-5-bromo-7-nitroindoline-3-yl)-1',2'-dideoxy-D-ribofuranose (22) Under an Ar atmosphere, **21** (202 mg, 0.569 mmol) was dissolved in TFA (1.1 mL) at 0°C. Then, NaNO_3 (53.2 mg, 0.626 mmol) were added, and the reaction was stirred at room temperature for 40 min. The reaction mixture was diluted with EtOAc and washed with aqueous saturated NaHCO_3 . The organic layer was dried with Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography on silica gel (AcOEt to AcOEt/ CHCl_3 /MeOH = 80:19:1) to obtain **22** (186.3 mg, 0.467 mmol, 82%) as a yellow foam. ^1H -NMR (500 MHz, CD_3OD) δ (ppm): 7.95 (0.5H, dd, $J=7.9, 1.5$ Hz), 7.86–7.85 (1H, m), 7.81 (0.5H, d, $J=7.8, 2.0$ Hz), 4.39–4.28 (3H, m), 4.23–4.16 (1H, m), 3.84 (0.5H, dt, $J=4.3, 2.3$ Hz), 3.76 (0.5H, dt, $J=4.6, 2.7$ Hz), 3.66–3.63 (1H, m), 3.56–3.48 (2H, m), 2.27 (1.5H, s), 2.26 (1.5H, s), 1.92 (0.5H, ddd, $J=13.0, 5.3, 1.8$ Hz), 1.84 (0.5H, ddd, $J=13.0, 5.5, 2.0$ Hz), 1.79 (0.5H, dd, $J=10.5, 6.0$ Hz), 1.77 (0.5H, dd, $J=10.8, 6.3$ Hz); ^{13}C -NMR (125 MHz, CD_3OD) δ (ppm): 171.4, 142.3, 141.8, 135.3, 134.2, 133.4, 126.7, 117.3, 89.1, 88.9, 81.3, 80.5, 73.9, 73.7, 63.8, 63.7, 53.8, 53.2, 45.7, 39.0, 23.2; ESI-HRMS (m/z) Calcd. for $\text{C}_{15}\text{H}_{17}\text{BrN}_2\text{O}_6\text{Na}$ [$\text{M} + \text{Na}$] $^+$: 423.0162, 425.0143, Found: 423.0179, 425.0162.

1' β -(*N*-Acetyl-7-nitroindoline-3-yl)-1',2'-dideoxy-D-ribofuranose (8) After three vacuum/ H_2 cycles to remove air from the round-bottom flask, a mixture of **22** (12.5 mg, 31.2 μmol), 10% Pd/C (1.3 mg, 12.5 μmol) and NaHCO_3 (7.9 mg, 93.6 μmol) in methanol (0.31 mL) was stirred at room temperature for 20 min under ambient pressure of hydrogen (balloon). The reaction mixture was filtered through a Celite pad, and the filtrate was evaporated. The residue was purified by flash chromatography on silica gel (CHCl_3 / CH_3CN = 2:1) to obtain **8** (5.4 mg, 16.8 μmol , 54%, $dr=50:50$) as a yellow oil. ^1H -NMR (500 MHz, CD_3OD) δ (ppm): 7.76–7.74 (0.5H, m), 7.69–7.68 (1H, m), 7.65–7.63 (1H, m), 7.28–7.24 (1H, m), 4.38–4.29 (2.5H, m), 4.24–4.17 (1.5H, m), 3.81 (0.5H, dt, $J=4.6, 2.5$ Hz), 3.76 (0.5H, dt, $J=4.5, 2.7$ Hz), 3.65–3.57 (1H, m), 3.52 (1H, t, $J=4.6$ Hz), 3.46 (1H, t, $J=4.5$ Hz), 2.27 (1.5H, s), 2.26 (1.5H, s), 1.93 (0.5H, ddd, $J=13.0, 5.3, 1.8$ Hz), 1.84–1.77 (1.5H, m); ^{13}C -NMR (125 MHz, CD_3OD) δ (ppm): 171.6, 171.5, 142.4, 142.4, 139.5, 139.3, 135.9, 135.8, 131.2, 130.5, 126.0, 124.1, 124.1, 89.0, 88.9, 81.5, 80.7, 74.0, 73.7, 63.8, 53.8, 53.6, 46.2, 46.1, 39.3, 38.4, 23.2, 23.2; ESI-HRMS (m/z) Calcd. for $\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}_6\text{Na}$ [$\text{M} + \text{Na}$] $^+$: 345.1057, Found: 345.1058.

1' β -(*N*-Acetyl-7-nitroindoline-3-yl)-1',2'-dideoxy-5'-*O*-(4,4'-dimethoxytriphenylmethyl)-D-ribofuranose-3'-[(2-Cyanoethyl)(*N,N*-diisopropyl)]-Phosphoramidite (23) Under an Ar atmosphere, **8** (26.4 mg, 81.9 μmol) was dried by azeotrope with CH_3CN and pyridine. The residue was dissolved in pyridine (0.33 mL). Then, DMTrCl (41.7 mg, 123 μmol) was added at 0°C, and the reaction was stirred at room temperature for 1 h. The reaction was quenched with aqueous saturated NaHCO_3 and extracted with AcOEt. The or-

ganic layer was washed with brine, dried with Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography on silica gel (hexane/EtOAc = 1:1 to 1:2) to obtain 5'-*O*-(4,4'-dimethoxytriphenylmethyl) derivative of **8** (34.5 mg, 55.7 μmol , 68%, $dr=50:50$) as a pale yellow foam. Each spectrum data of diastereomers were shown separately. 1) ^1H -NMR (500 MHz, CDCl_3) δ (ppm): 7.65 (1H, d, $J=8.0$ Hz), 7.61 (1H, d, $J=7.8$ Hz), 7.42–7.40 (2H, m), 7.30–7.27 (6H, m), 7.24–7.21 (1H, m), 7.10 (1H, dd, $J=8.0, 7.8$ Hz), 6.84–6.81 (4H, m), 4.39–4.35 (2H, m), 4.23–4.19 (1H, m), 4.01 (1H, dd, $J=11.0, 5.5$ Hz), 3.97 (1H, ddd, $J=4.9, 4.5, 2.3$ Hz), 3.79 (6H, s), 3.67–3.63 (1H, m), 3.29 (1H, dd, $J=9.6, 4.5$ Hz), 3.12 (1H, dd, $J=9.6, 4.9$ Hz), 2.08 (3H, s), 1.83–1.81 (2H, m); ^{13}C -NMR (125 MHz, CD_3OD) δ (ppm): 168.6, 158.7, 144.8, 141.0, 137.3, 135.9, 135.9, 134.9, 130.2, 130.2, 129.7, 128.3, 128.0, 127.1, 124.6, 123.6, 113.3, 86.6, 86.4, 79.9, 74.0, 64.3, 55.4, 52.1, 45.0, 38.1, 23.3; ESI-HRMS (m/z) Calcd. for $\text{C}_{36}\text{H}_{36}\text{N}_2\text{O}_8\text{Na}$ [$\text{M} + \text{Na}$] $^+$: 647.2364, Found: 647.2334. 2) ^1H -NMR (500 MHz, CDCl_3) δ (ppm): 7.65 (1H, d, $J=8.1$ Hz), 7.43–7.39 (3H, m), 7.30–7.27 (6H, m), 7.24–7.20 (1H, m), 7.06 (1H, dd, $J=8.1, 7.8$ Hz), 6.84–6.82 (4H, m), 4.35–4.30 (2H, m), 4.23 (2H, d, $J=7.0$ Hz), 3.94–3.91 (1H, m), 3.80 (3H, s), 3.79 (3H, s), 3.54 (1H, dd, $J=13.0, 6.5$ Hz), 3.25 (1H, dd, $J=9.9, 4.3$ Hz), 3.07 (1H, dd, $J=9.9, 5.5$ Hz), 2.13 (3H, s), 1.93 (1H, ddd, $J=12.8, 5.3, 1.8$ Hz), 1.78 (1H, ddd, $J=12.8, 10.1, 6.1$ Hz); ^{13}C -NMR (125 MHz, CD_3OD) δ (ppm): 168.7, 158.7, 144.8, 141.0, 136.6, 136.0, 135.8, 135.2, 130.2, 130.2, 129.2, 128.2, 128.0, 127.1, 124.6, 123.7, 113.3, 86.5, 86.2, 78.9, 74.4, 64.2, 55.4, 52.9, 45.3, 38.2, 23.3; ESI-HRMS (m/z) Calcd. for $\text{C}_{36}\text{H}_{36}\text{N}_2\text{O}_8\text{Na}$ [$\text{M} + \text{Na}$] $^+$: 647.2364, Found: 647.2385.

Under an Ar atmosphere, the above compound (34.5 mg, 55.2 μmol) was dried by azeotrope with CH_3CN and dissolved in CH_2Cl_2 (0.50 mL). Then, DIPEA (58 μL , 331 μmol) and 2-cyanoethyl *N,N*-diisopropylchloro-phosphoramidite (37 μL , 166 μmol) were added, and stirred at 0°C for 1 h. The reaction was quenched with aqueous saturated NaHCO_3 and extracted with CH_2Cl_2 . The organic layer was washed with brine, dried with Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography on silica gel (hexane/EtOAc = 2:1) to obtain the material, which was crystallized in hexane at -78°C. The hexane was removed by decantation, and the solid material was dried in a vacuum to give **23** (31 mg, 37.5 μmol , 68%, $dr=50:50$) as a yellow foam. ^1H -NMR (500 MHz, CD_3OD) δ (ppm): 7.67–7.65 (1H, m), 7.47–7.41 (3H, m), 7.28–7.27 (6H, m), 7.24–7.21 (1H, m), 7.13–7.05 (1H, m), 6.84–6.81 (4H, m), 4.53–4.50 (0.5H, m), 4.47–4.43 (0.5H, m), 4.36–4.21 (3.0H, m), 4.16–4.14 (0.5H, m), 4.11–4.10 (0.5H, m), 3.80 (3H, s), 3.79 (3H, s), 3.73–3.63 (2.0H, m), 3.59–3.51 (3H, m), 3.24–3.08 (2H, m), 2.58 (1H, d, $J=6.3$ Hz), 2.44 (0.5H, t, $J=6.5$ Hz), 2.43 (0.5H, t, $J=6.3$ Hz), 2.14–2.09 (3.5H, m), 2.04–2.00 (0.25H, m), 1.95–1.92 (0.25H, m), 1.89–1.75 (1.0H, m), 1.17–1.14 (6.0H, m), 1.11–1.06 (6.0H, m); ^{31}P -NMR (202 MHz, CDCl_3) δ (ppm): 148.2, 148.1, 148.0; ESI-HRMS (m/z) Calcd. for $\text{C}_{45}\text{H}_{53}\text{N}_4\text{O}_9\text{PNa}$ [$\text{M} + \text{Na}$] $^+$: 847.3442, Found: 847.3460.

3-Bromoacetyl-7-nitroindole (24) Under an Ar atmosphere, 7-nitroindole (200 mg, 1.357 mmol) was dissolved in dry dichloroethane (14.6 mL), followed by the addition of aluminium chloride (410.7 mg, 2.46 mmol) and bromoacetyl chloride (205 μL , 2.46 mmol) at 0°C, and the reaction mixture was stirred at 80°C for 2 h. The reaction mixture was

quenched with water. The mixture was filtered off and the filtrate was evaporated to dryness. The residue was purified by flash chromatography on silica gel (hexane/EtOAc = 9:1 to 3:1 to 2:1) to obtain **24** (227.2 mg, 0.882 mmol, 65%) as a red amorphous solid. ¹H-NMR (500 MHz, CDCl₃) δ (ppm): 10.38 (1H, br), 8.79 (1H, d, J = 8.0 Hz), 8.26 (1H, d, J = 8.0 Hz), 8.16 (1H, d, J = 3.0 Hz), 7.45 (1H, dd, J = 8.0, 8.0 Hz), 4.34 (2H, s); ¹³C-NMR (125 MHz, CDCl₃) δ (ppm): 187.0, 133.6, 131.0, 129.8, 129.1, 122.9, 121.2, 115.8, 31.4; IR (neat): 1666, 1532 cm⁻¹; ESI-HRMS (m/z) Calcd. for C₁₀H₇BrN₂O₃Na [M + Na]⁺: 304.9532, 306.9512, Found: 304.9531, 306.9515.

(2R)-3-(2-(7-Nitroindolin-3-yl)ethoxy)propane-1,2-diol (26) Under an Ar atmosphere, a mixture of (S)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol (87 μ L, 0.706 mmol) and NaH (60%, 50.8 mg, 1.27 mmol) in THF (0.88 mL) was stirred at room temperature for 1 h. Then, a solution of **24** (100 mg, 0.353 mmol) in THF (1.76 mL) was added and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with CH₂Cl₂, washed with aqueous saturated NaHCO₃ and brine and dried with Na₂SO₄. The organic layer was evaporated to dryness under an Ar atmosphere, and the residue was dissolved in trifluoroacetic acid (1.7 mL) at 0 °C. Then, sodium cyanoborohydride (221.8 mg, 3.53 mmol) was added and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with MeOH and evaporated to dryness. The residue was purified by flash chromatography on silica gel (CHCl₃/MeOH = 1:0 to 12:1) to obtain **26** (78.8 mg, 0.279 mmol, 79%, 2steps, dr = 50:50) as an orange oil. ¹H-NMR (500 MHz, CDCl₃) δ (ppm): 7.81 (1H, d, J = 9.0 Hz), 7.19 (0.5H, d, J = 7.0 Hz), 7.19 (0.5H, d, J = 7.0 Hz), 6.60 (0.5H, dd, J = 9.0, 7.0 Hz), 6.59 (0.5H, dd, J = 9.0, 7.0 Hz), 3.99 (2H, t, J = 9.0 Hz), 3.91–3.87 (1H, m), 3.74 (1H, dd, J = 11.0, 4.0 Hz), 3.66–3.63 (1H, m), 3.61–3.50 (6H, m), 2.11–2.04 (1H, m), 1.93–1.86 (1H, m); ¹³C-NMR (125 MHz, CDCl₃) δ (ppm): 148.7, 137.0, 129.4, 123.0, 116.6, 72.7, 72.7, 70.7, 69.2, 69.2, 64.2, 53.2, 53.2, 38.1, 38.1, 34.7; IR (neat): 3432, 1626, 1592, 1511, 1443, 1402, 1327 cm⁻¹; ESI-HRMS (m/z) Calcd. for C₁₃H₁₈N₂O₅ [M + Na]⁺: 305.1108, Found: 305.1110.

(2R)-3-(2-(N-Acetyl-7-nitroindolin-3-yl)ethoxy)-propane-1,2-diol (9) Under an Ar atmosphere, **26** (240.2 mg, 0.851 mmol) was dissolved in dry dichloroethane (10.4 mL). Then, aluminum chloride (284.0 mg, 2.13 mmol) and acetyl chloride (243 μ L, 3.40 mmol) were added at 0 °C and the reaction mixture was stirred at 80 °C for 40 min. The reaction mixture was quenched with MeOH and evaporated to dryness. The residue was purified by flash chromatography on silica gel (CHCl₃/MeOH = 1:0 to 19:1 to 10:1) to obtain **9** (202.7 mg, 0.621 mmol, 73%, dr = 50:50) as an orange oil. ¹H-NMR (500 MHz, CDCl₃) δ (ppm): 7.66 (1H, d, J = 8.0 Hz), 7.40 (0.5H, d, J = 7.8 Hz), 7.39 (0.5H, d, J = 7.8 Hz), 7.18 (1H, dd, J = 8.0, 7.8 Hz), 4.37 (0.5H, dd, J = 8.8, 2.5 Hz), 4.35 (0.5H, dd, J = 8.5, 2.3 Hz), 4.01 (0.5H, dd, J = 5.8, 2.5 Hz), 3.99 (0.5H, dd, J = 5.5, 2.3 Hz), 3.88–3.84 (1H, m), 3.69 (1H, dt, J = 11.4, 3.8 Hz), 3.61–3.46 (6H, m), 2.13–2.04 (1H, m), 1.91–1.85 (1H, m); ¹³C-NMR (125 MHz, CDCl₃) δ (ppm): 169.1, 141.0, 140.3, 134.3, 128.2, 124.9, 123.1, 72.6, 70.8, 69.1, 69.0, 64.0, 64.0, 56.5, 56.4, 39.0, 39.0, 33.8, 23.4; ESI-HRMS (m/z) Calcd. for C₁₅H₂₀N₂O₆Na [M + Na]⁺: 347.1214, Found: 347.1227.

N-Acetyl-3-((2S)-3-(4,4'-dimethoxytriphenyl-methoxy)-2-((2-cyanoethoxy)(N,N-diisopropylamino)phosphinyl)-

propoxy)ethyl)-7-nitroindoline (27) Under an Ar atmosphere, **9** (202.7 mg, 0.625 mmol) was dried by azeotrope with pyridine. The residue was dissolved in pyridine (2.5 mL). Then, *N,N*-dimethyl-4-aminopyridine (7.6 mg, 62.5 μ mol) and DMTrCl (233.1 mg, 0.688 mmol) were added at 0 °C, and the reaction was stirred at room temperature for 6 h. The reaction mixture was evaporated to dryness. The residue was purified by flash chromatography on silica gel (hexane/EtOAc = 2:1 to 1:2 to 1:4) to obtain the 4,4'-dimethoxytriphenylmethoxy derivative of **9** (203.9 mg, 0.325 mmol, 52%, dr = 50:50) as a pale yellow foam. ¹H-NMR (500 MHz, CDCl₃) δ (ppm): 7.66 (1H, d, J = 8.0 Hz), 7.43–7.41 (1H, m), 7.36–7.34 (1H, m), 7.31–7.28 (6H, m), 7.22–7.20 (1H, m), 7.16–7.12 (1H, m), 6.83–6.80 (4H, m), 4.26 (1H, dd, J = 11.0, 8.5 Hz), 3.96–3.89 (2H, m), 3.77 (3H, s), 3.76 (3H, s), 3.57–3.51 (4H, m), 3.46–3.42 (1H, m), 3.21 (2H, t, J = 5.0 Hz), 2.20 (1.5H, s), 2.19 (1.5H, s), 2.03–1.98 (1H, m), 1.83–1.76 (1H, m); ¹³C-NMR (125 MHz, CDCl₃) δ (ppm): 168.6, 168.6, 158.7, 144.8, 141.1, 140.2, 136.0, 134.4, 130.2, 128.2, 128.2, 128.1, 128.0, 127.1, 124.7, 123.2, 113.3, 86.4, 72.6, 72.6, 70.2, 69.1, 69.0, 64.6, 56.5, 56.5, 55.4, 39.0, 34.1, 23.3; IR (neat): 1682, 1607, 1537, 1509 cm⁻¹; ESI-HRMS (m/z) Calcd. for C₃₆H₃₈N₂O₈Na [M + Na]⁺: 649.2520, Found: 649.2521.

Under an Ar atmosphere, the above compound (100 mg, 0.16 mmol) was dried by azeotrope with CH₃CN and dissolved in CH₂Cl₂ (1.4 mL). Then, DIPEA (167 μ L, 0.96 mmol) and 2-cyanoethyl *N,N*-diisopropylchloro-phosphoramidite (107 μ L, 0.48 mmol) were added, and stirred at 0 °C for 1 h. The reaction was quenched with aqueous saturated NaHCO₃ and extracted with CH₂Cl₂. The organic layer was washed with brine, dried with Na₂SO₄ and evaporated to dryness. The residue was purified by flash chromatography on silica gel (hexane/EtOAc = 2:1 to 1:1) to obtain the material, which was crystallized in hexane at –78 °C. The hexane solution was removed by decantation, and the solid material was dried in a vacuum to give **27** (109.8 mg, 0.133 mmol, 83%, dr = 50:50) as a yellow foam. ¹H-NMR (500 MHz, CDCl₃) δ (ppm): 7.65 (1H, d, J = 8.0 Hz), 7.46–7.44 (2H, m), 7.35–7.30 (5H, m), 7.28–7.24 (2H, m), 7.21–7.17 (1H, m), 7.14–7.12 (1H, m), 6.81–6.78 (4H, m), 4.23–4.11 (2H, m), 3.90–3.84 (2H, m), 3.80–3.75 (7H, m), 3.73–3.68 (1H, m), 3.66–3.50 (5H, m), 3.41–3.33 (1H, m), 3.31–3.23 (1.5H, m), 3.19–3.15 (0.5H, m), 2.62 (2H, t, J = 6.3 Hz), 2.47–2.44 (1H, m), 2.17 (1.5H, s), 2.16 (1.5H, s), 1.98–1.91 (1H, m), 1.79–1.69 (1H, m), 1.20–1.18 (6H, m), 1.16 (3H, d, J = 6.5 Hz), 1.09 (1.5H, d, J = 7.0 Hz), 1.08 (1.5H, d, J = 7.0 Hz); ³¹P-NMR (202 MHz, CDCl₃) δ (ppm): 149.3, 149.3, 149.4, 149.4; ESI-HRMS (m/z) Calcd. for C₄₅H₅₅N₄O₉PNa [M + Na]⁺: 847.3599, Found: 847.3592.

Synthesis and Purification of Oligodeoxynucleotides

All the oligonucleotides were synthesized on a 1 μ mol scale by standard β -cyanoethyl phosphoramidite chemistry. The 5'-terminal dimethoxytrityl-bearing ODN was removed from the solid support by treatment with 45 mM K₂CO₃ in dry MeOH (1 mL). After 1 h, 0.1 M TEAA buffer (1 mL) was added to the solution, and the mixture was purified by reverse-phase HPLC (Column: SHISEIDO C18, 4.6 $\times$ 250 mm, Solvent A: 0.1 M TEAA Buffer, Solvent B: CH₃CN, B: 10 to 40% /20 min, linear gradient; Column oven: 35 °C; Flow rate: 1.0 mL/min; monitored by UV detector at 254 nm). The dimethoxytrityl group of the purified ODN was cleaved with 5% AcOH and the mixture was additionally purified by HPLC (for ODN1;

Column: SHISEIDO C18, 4.6×250 mm, Solvent A: 0.1 M TEAA buffer, Solvent B: CH_3CN , B: 10 to 14% /20 min, linear gradient; Column oven: 35°C ; Flow rate: 1.0 mL/min; monitored by UV detector at 254 nm, for ODN2, 3; Column: SHISEIDO C18, 4.6×250 mm, Solvent A: 0.1 M TEAA Buffer, Solvent B: CH_3CN , B: 10 to 18% /20 min, linear gradient; Column oven: 35°C ; Flow rate: 1.0 mL/min; monitored by UV detector at 254 nm). The MALDI-TOF/MS data are summarized in Table S2.

Photoreaction The reaction between ODN1 and RNA1(C) is described as a general procedure. The mixture of ODN1 ($5\ \mu\text{M}$) and RNA1(C) ($5\ \mu\text{M}$) in phosphate buffer (50 mM) containing 100 mM NaCl at pH 7 in a polypropylene microtube was irradiated at room temperature using a 4 W UVGL-25 Compact UV lamp ($\lambda = 365$ nm). The UV lamp was placed 15 cm above the microtube. The reaction mixture was analyzed by reverse-phase HPLC (Column: SHISEIDO C18, 4.6×250 mm, Solvent A: 0.1 M TEAA Buffer, Solvent B: CH_3CN , B: 10 to 15% /20 min, linear gradient; Column oven: 35°C ; Flow rate: 1.0 mL/min; monitored by UV detector at 254 nm. The results are summarized in Fig. 2.

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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