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Syntheses of pyrido[1,2-*a*][1,3,5]triazin-4-one C-deoxyribonucleosides

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Abstract—We achieved the syntheses of new C-deoxyribonucleosides bearing pyrido[1,2-*a*][1,3,5]triazin-4-one derivatives using palladium-catalyzed Heck-type coupling. Some of these C-deoxyribonucleosides were able to convert to phosphoramidite reagents, which can be used for DNA synthesizer. DNA oligomers including pyrido[1,2-*a*][1,3,5]triazin-4-one C-deoxyribonucleoside, which had 2-amino group were synthesized, and T_m values with a natural nucleoside were measured.

1. Introduction

DNA that consists of the four bases A, G, C, and T serves as one of the most important biomolecules by coding indispensable information for life. An important function of DNA for the transmittance of genes is replication. A new aptamer that especially interacts with a specific biomolecule has recently been well investigated.[1] However, it seems to be impossible to prepare all aptamers with satisfactory levels of specificity. Because an aptamer is a small nucleic acid compared with a general target such as a protein, there is a limitation in the diversity. A new nucleoside pair that can replicate by polymerase chain reaction (PCR) is being investigated.[2] If this succeeds, the genetic alphabet will increase. If the newly added codon can be assigned to unnatural amino acids, it might be possible to synthesize a functionalized protein in vitro or in vivo. Of course, several further issues must be solved before this can be realized.

Unlike natural A–T and G–C pairs, the newly added nucleoside includes a base pair utilizing the shape-fitting and hydrophobic interaction. Hirao et al. reported a successful DNA replication using such kind of base pair. However, when introducing a large amount of this unnatural base pair, the nucleic acid containing this base pair is expected to cohere and take an irregular structure, even if it replicates correctly when using only one base pair. Moreover, it is thought to be difficult to use this type of base pair for a codon, since the codon consists of only three bases. The binding energy of the hydrophobic interaction is significantly weak compared with that of the hydrogen bond. Therefore there is a possibility that complementary tRNA cannot be accurately selected.

On the other hand, when designing an artificial nucleoside utilizing a hydrogen bond similar to the natural nucleoside, the artificial nucleoside must have the different directions of the hydrogen bonds from natural four bases. At the same time, chemical stability against the tautomerization that breaks down the designed hydrogen bonding pattern is important. To our knowledge, known artificial base pairs using hydrogen bonds do not have very high selectivity similar to the natural bases so far, thus there is no report about replication of DNA containing these nucleoside pairs under the typical conditions of PCR.

We synthesized pyrido[1,2-*a*][1,3,5]triazin-4-one nucleoside derivatives that have the possibility for such purpose. There are many reports about the construction of the pyrido[1,2-*a*][1,3,5]triazin-4-one ring that has various substituents. This heterocycle has a rigid planar structure, and cannot be subjected to tautomerization. Hitherto, pyrido[1,2-*a*][1,3,5]triazin-4-one has not be employed as a base for artificial nucleoside. Because this heterocycle does not form complemented hydrogen bond with natural base, it is promising as a counterpart of an artificial base that can replicate by PCR.

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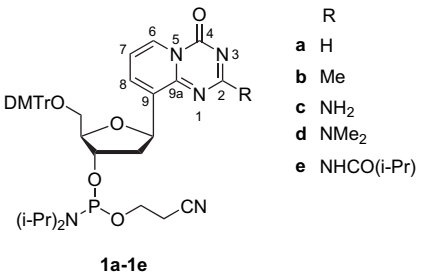


Figure 1. Target molecule.

We examined the syntheses of the nucleoside amidites **1a**–**1e**, which had a pyrido[1,2-*a*][1,3,5]triazin-4-one skeleton, and synthesized DNA oligomer including **1c** or **1d** (Fig. 1). These nucleosides do not only have a different hydrogen bond direction compared to the natural bases but also unlike natural purine bases, attached with the deoxyribose moiety through the six-membered ring. They are therefore expected to effectively inhibit the base pair formation of the pyrimidine bases. The syntheses of the oligomers containing the new artificial C-deoxyribonucleoside and their properties are described.

2. Results and discussion

2.1. Syntheses of pyrido[1,2-*a*][1,3,5]triazin-4-one nucleoside phosphoramidites

Synthesis of the pyrido[1,2-*a*][1,3,5]triazin-4-one skeleton was achieved using 2-amino-3-iodopyridine as the starting material.[3a] When the substituent on the 2-position of pyrido[1,2-*a*][1,3,5]triazin-4-one belonged to the hydrogen or alkyl group (compounds **3a** and **3b**), 2-amino-3-iodopyridine was reacted with *ortho*-ester,[4] followed by treatment with trimethylsilyl isocyanate. Moreover, in the case of 2-amino substituted pyrido[1,2-*a*]-[1,3,5]triazin-4-one (compounds **3c** and **3d**), 2-amino-3-iodopyridine was converted to thiourea by treatment with ethoxycarbonyl isothiocyanate. Thiourea was treated with mercury(II) salt as a scavenger in the presence of amine, and the annulation of the resulting guanidine was carried out by heating or by the reaction of hydrogen chloride.

The synthesis of pyrido[1,2-*a*][1,3,5]triazin-4-one nucleoside phosphoramidite is shown in Scheme 1. At first, we tried to synthesize the non-substituted C-deoxyribonucleoside at the 2-position of the triazine ring. However, the Heck-type palladium-catalyzed reaction between 9-iodopyrido[1,2-*a*][1,3,5]triazin-4-one **3a** with silyl protected furanoid glycal **10**[5] gave a complex mixture and the desired coupling product was not detected. On the other hand, the Heck coupling reaction using 2-methyl derivative of 9-iodopyrido[1,2-*a*][1,3,5]triazin-4-one **3b** succeeded to give the adduct **5b**, which is a hydrolyzed product of 3′-silyl enol ether. Although the Heck coupling reaction of iodide **3a** was not successful, the synthesis of the corresponding non-substituted adduct **6a** was achieved by replacing the order of annulation and Heck reaction. Adduct **4** was obtained through the reaction of 2-amino-3-iodopyridine **2** with **10** in the presence of palladium catalyst in moderate yield. The formation of the pyrido[1,2-*a*][1,3,5]triazin-4-one ring was carried out using triethyl orthoformate and trimethylsilyl isocyanate.

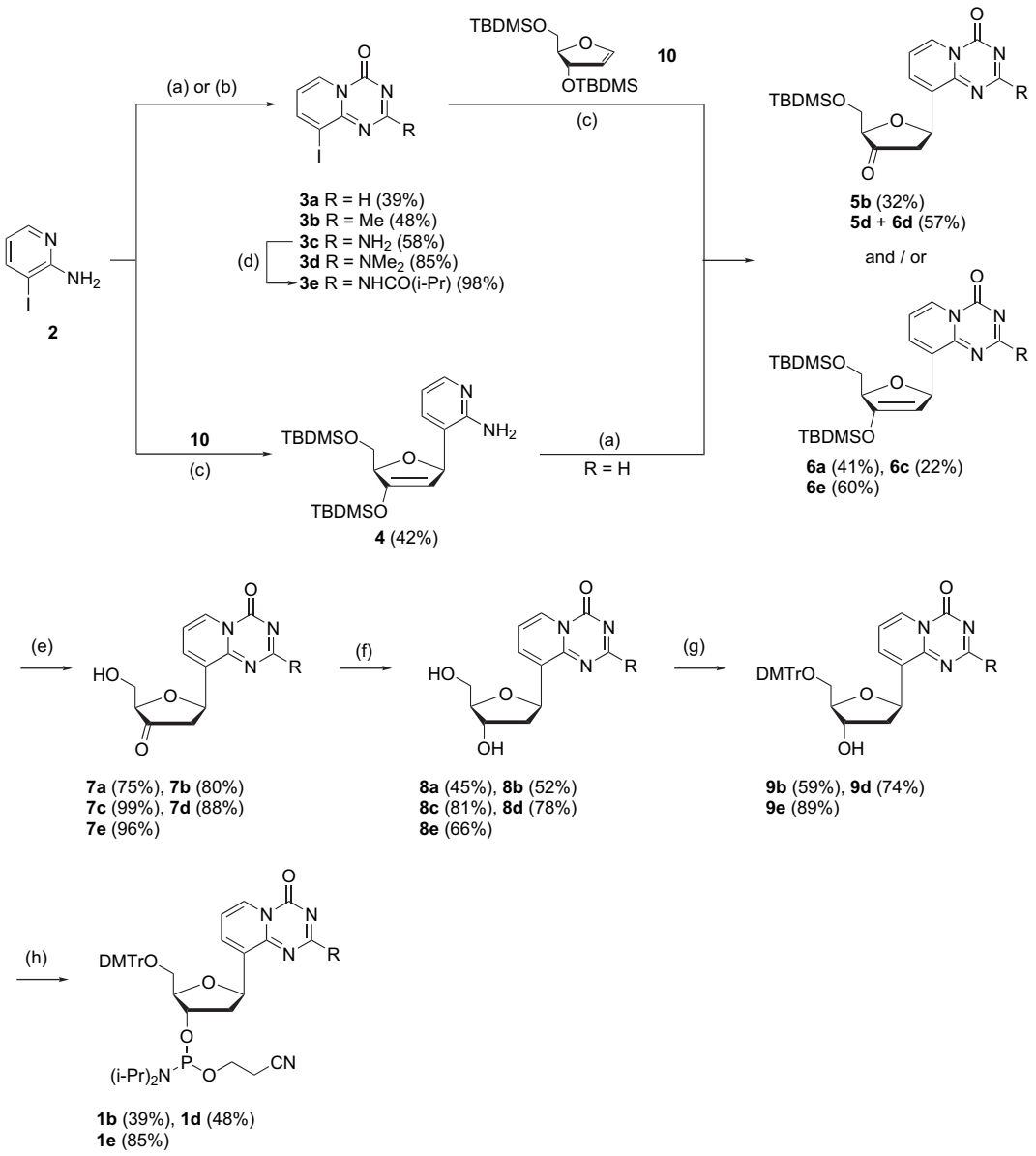
2-Amino derivatives of 9-iodopyrido[1,2-*a*][1,3,5]triazin-4-one were synthesized as follows. The reaction of amine **2** with ethoxycarbonyl isothiocyanate and the subsequent one-pot reaction using ammonia in the presence of mercury(II) salt gave guanidine derivative. 2-Amino-9-iodopyrido[1,2-*a*][1,3,5]triazin-4-one (**3c**) was obtained by heating the DMF solution of the intermediate guanidine derivative in a microwave instrument. The Heck reaction of **3c** with **10** gave a somewhat low yield. However, it is necessary to introduce a protecting group on the free amino group for the synthesis of DNA oligomer. Thus, we examined the Heck reaction using *N*-protected 2-amino-9-iodopyrido[1,2-*a*][1,3,5]triazin-4-one (**3e**) that was easily prepared through a reaction of **3c** with isobutyryl chloride. The yield of the Heck coupling adduct was significantly increased using **3e**.

The optimization of the reaction condition for the palladium-catalyzed Heck coupling between **3e** and **10** was performed for ligands, bases, solvents, and additives (Table 1). Comparably bulky ligands such as tri(*o*-tolyl)phosphine or tri(*tert*-butyl)phosphine were effective, whereas the adduct was not obtained using triphenylphosphine, triphenylarsine and tri(2-furyl)phosphine. Diisopropylethylamine gave a higher yield than triethylamine or dicyclohexylmethylamine. Inorganic bases such as sodium carbonate, potassium carbonate, cesium carbonate, silver carbonate, sodium acetate, and potassium phosphate were not effective in this coupling reaction. The coupling reaction occurred in acetonitrile and DMF but not in solvents such as THF, 1,4-dioxane, DMSO, and NMP. The reactions were examined in the presence of several ammonium salts and of lithium chloride as an additive, but none of the additives could be shown to be effective (data not shown).

Similar to the synthesis of **3c**, iodide **3d** was prepared using dimethylamine instead of ammonia. The annulation was carried out by refluxing the 1,4-dioxane solution of the intermediate guanidine derivative saturated with hydrogen chloride. The Heck reaction of **3d** proceeded smoothly and gave the corresponding adduct **6d** along with the 3′-desilylated product **5d** in good yield.

The products of the Heck reaction were subjected to deprotection by tetrabutylammonium fluoride in order to obtain 3′-keto nucleosides **7a**–**7e** in good to excellent yields. Compounds **7a**–**7e** were diastereoselectively reduced to pyrido[1,2-*a*][1,3,5]triazin-4-one C-deoxyribonucleosides **8a**–**8e** using sodium tri(acetoxy)borohydride.[6] The structures of **8a**–**8e** were determined by various spectra and was confirmed to have a β-anomer structure through an NOE experiment.

We have achieved the syntheses of some pyrido[1,2-*a*][1,3,5]triazin-4-one nucleoside derivatives (**8a**–**8e**) with 2-amino-3-iodopyridine as the starting material. The total yields of these artificial nucleosides were about 5–30%,



Scheme 1. Reagents and conditions: (a) (i) $RC(OEt)_3$, reflux, overnight; (ii) Me_3SiNCO, rt, two days; (b) (i) EtO_2CNCS, DMF, rt, 2 h; (ii) 0.5 M NH_3 in 1,4-dioxane or $HNMe_2 \cdot HCl$, $HgCl_2$, 0 °C, 15 min, then rt, 4 h; (iii) DMF, microwave 160 °C, 800 s or satd. HCl in 1,4-dioxane, reflux, 4 h; (c) **10**, Pd_2dba_3–$CHCl_3$, P(*t*-Bu)$_3$, *i*-Pr_2NEt, DMF, microwave 160 °C, 10 min; (d) *i*-PrCOCl, pyridine, 115 °C, 2 h; (e) TBAF, HOAc, THF, rt, overnight; (f) $NaBH(OAc)_3$, HOAc, MeCN, −40 °C, 30 min then 0 °C, 2 h; (g) DMTrCl, *i*-Pr_2NEt, pyridine, rt, 1 h; (h) $NCCH_2CH_2OP[N(i\text{-}Pr)_2]_2$, 1*H*-tetrazole, $HN(i\text{-}Pr)_2$, CH_2Cl_2, rt, 3 h.

Table 1. Optimization of the Heck reaction between **3e** and **10**[a]

Entry	Ligand	Base	Solvent	Isolated yield of **6e** (%)
1	PPh_3	(*i*-Pr)$_2$NEt	DMF	0
2	$AsPh_3$	(*i*-Pr)$_2$NEt	DMF	10
3	P(*t*-Bu)$_3$	(*i*-Pr)$_2$NEt	DMF	60
4	P(*o*-tolyl)$_3$	(*i*-Pr)$_2$NEt	DMF	49
5	P(*t*-Bu)$_3$	(*i*-Pr)$_2$NEt	THF	0
6	P(*t*-Bu)$_3$	(*i*-Pr)$_2$NEt	NMP	0
7	P(*t*-Bu)$_3$	(*i*-Pr)$_2$NEt	CH_3CN	38
8	P(*t*-Bu)$_3$	NEt_3	DMF	36
9	P(*t*-Bu)$_3$	Cy_2NMe	DMF	35
10	P(*t*-Bu)$_3$	Cs_2CO_3	DMF	0

[a] All reactions were carried out using **3e** (1 mmol), **10** (1 mmol), base (4.5 equiv), tris(dibenzylideneacetone)dipalladium(0) chloroform adduct (10 mol %), and ligand (20 mol %) in solvent (5 mL) under the irradiation of microwave at 160 °C for 10 min.

and the key steps were a triazine ring formation and a palladium-catalyzed Heck coupling reaction.

To synthesize the DNA oligomers containing these artificial nucleosides, we induced **8b**, **8d**, and **8e** to a phosphoramidite reagent. Unfortunately, for **8a** it was proved that the addition of alcohol occurred gradually at the 2,3-CN double bond by measuring of the ^{1}H NMR spectrum in methanol-d_4. Protic solvent is generally used for handling DNA, so it is difficult to keep the reactive enone structure. Thus, tritylation of **8a** was not performed. A reaction with nucleosides **8b**, **8d**, **8e**, and 4,4′-dimethoxytrityl chloride was carried out to obtain a corresponding tritylated product in good to moderate yields. The desired phosphoramidite reagents for DNA synthesizers **1b**, **1d**, and **1e** were obtained through a reaction with

2-cyanoethyl phosphordiamidite in the presence of 1*H*-tetrazole.

2.2. Thermal denaturation studies

Oligonucleotide was synthesized using nucleoside phosphoramidites **1b**, **1d**, and **1e** synthesized through the method described above. All oligonucleotides containing these artificial nucleosides could be synthesized under the conventional condition of a DNA synthesizer. The trityl monitor indicated that the extension reaction of **1b**, **1d**, and **1e** proceeded with almost the same efficiency as the phosphoramidite reagents bearing a natural base. The oligonucleotide attached CPG column was treated with ammonia at 55 °C overnight, and tritylated oligonucleotide was purified and deprotected by reverse-phase cartridge. Further purification was performed by HPLC using ion-exchange resin. To our regret, it was confirmed through the ESI-TOF mass spectrum that the oligonucleotide containing nucleoside **8b** decomposed to 2-aminopyridine at the base moiety. While **8b** was not subjected to the Michael-type addition at the stage of the nucleoside, it was decomposed under strong basic condition at the deprotection step of the synthesized oligonucleotide. We assume that the ring-opening reaction and the following hydrolysis occurred at the triazine ring through ammonia treatment of CPG. The content of the artificial nucleosides **8c** and **8d** was confirmed by the ESI-TOF mass spectra. Pyrido[1,2-*a*][1,3,5]triazin-4-one possessing the 2-amino group could be synthesized at comparably high yield and was stable under the basic condition.

The measurement of the T_m values between the natural base and the pyrido[1,2-*a*][1,3,5]triazin-4-one base are shown in Table 2. Samples were kept at least 2 min at 10 °C and were then heated from 10 °C to 70 °C at a rate of 0.5 °C/min. The absorbance at 260 nm was measured every 10 s. Each oligonucleotide pair containing **8c** and **8d** gave low T_m values compared with the correct natural base pair. This result shows that these artificial nucleosides do not efficiently form base pairs with natural bases.

Although thermal denaturation studies for the duplex DNA containing **8c** or **8d** clearly indicate the inefficient base pairing between these artificial nucleosides and all four natural bases, **8c** showed higher T_m value against cytidine in contrast to **8d**. We assume that the miss-annealing occurred in using

Table 2. Thermal denaturation studies[a]

X/Y	T_m (°C)	ΔT_m (°C)
T/A	45.5	
C/G	47.2	1.7
A/**8c**	32.7	−12.8
G/**8c**	32.2	−13.3
C/**8c**	40.4	−5.1
T/**8c**	36.8	−8.7
A/**8d**	32.6	−12.9
G/**8d**	32.6	−12.9
C/**8d**	33.9	−11.6
T/**8d**	32.3	−13.2

Sequence pair
5′-GGTAAC-**X**-ATGCG-3′
3′-CCATTG-**Y**-TACGC-5′.

[a] Condition: 0.17 mM purified oligonucleotide, 10 mM Na_3PO_4, 100 mM NaCl, and 0.1 mM EDTA at pH 7.0.

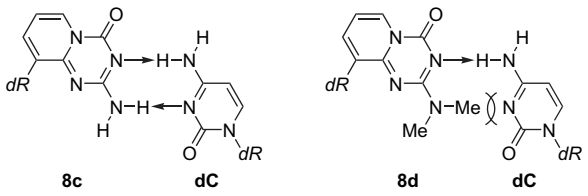


Figure 2. Slippage of **8c** and steric effect of **8d**.

8c as shown in Figure 2. On the other hand, a low T_m value similar to other bases was obtained in **8d** due to a steric hindrance of the 2-dimethylamino group introduced to the triazine ring. This result is an evidence for the existence of miss-annealing by slippage.[7]

3. Conclusion

We synthesized the artificial nucleosides possessing 6–6 fusion ring with particular hydrogen bond pattern, and also achieved the syntheses of the oligonucleotide containing these nucleoside. A five- or six-membered ring having lined two proton donor sites is a complementary nucleoside to the pyrido[1,2-*a*][1,3,5]triazin-4-one nucleoside. The syntheses of such nucleosides are in progress.

4. Experimental

4.1. General

^{1}H NMR spectra were recorded on a JEOL JNM-α400 (400 MHz) instrument. The chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane (δ=0 ppm). The signal patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br s, broad singlet. The coupling constants (J) are given in hertz. The ^{13}C NMR spectra were recorded on a JEOL JNM-α400 (100 MHz) instrument. The chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane (δ=0 ppm). ^{31}P NMR spectra were recorded on a JEOL JNM-α400 (160 MHz) instrument. The chemical shifts are reported in parts per million (δ) downfield from external 85% phosphoric acid (δ=0 ppm). FABMS spectra were measured on a JEOL JMS-700 instrument and are reported in the order of the molecular ion peak and remarkable high peaks (intensity). ESI-TOF mass spectra were measured on a JEOL JMS-T100LC instrument. Preparative and analytical HPLC were performed with a Shimadzu LC-6A instrument using a TOSOH TSK-gel DNA-NPR column. The reactions involving heating in a microwave were carried out using a Biotage Initiator Sixty instrument. The products were purified using the flash chromatography technique on silica gel 60 (40–100 μm) purchased from Kanto Chemical Co., Inc. Commercial grade reagents and solvents were used as supplied. Furanoid glycal **10** was prepared according to the method described in the literature.[4] All reactions sensitive to oxygen or moisture were carried out under nitrogen atmosphere.

4.2. 2-Amino-3-iodopyridine (2)[8]

A mixture of 2-amino-3-bromopyridine (17.3 mmol, 3.00 g), sodium iodide (34.6 mmol, 5.19 g), copper(I) iodide

(5 mol %, 0.165 g), and *trans*-*N*,*N*′-dimethylcyclohexane-1,2-diamine (10 mol %, 0.246 g) in 1,4-dioxane (36 mL) was stirred at 110 °C for 24 h. The mixture was poured into water and extracted with ether. The combined organic layer was washed with brine, dried, and concentrated. The product was isolated by flash column chromatography (*n*-hexane–ethyl acetate=1:1, R_f=0.45). Yield 86% (3.29 g, light gray powder). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (dd, J=1.0, 4.9 Hz, 1H), 7.86 (dd, J=1.5, 7.8 Hz, 1H), 6.40 (dd, J=4.9, 7.8 Hz, 1H), 4.97 (br s, 2H).

4.3. 9-Iodopyrido[1,2-*a*][1,3,5]triazin-4-one (3a)

A mixture of **2** (8 mmol, 1.76 g) and triethyl orthoformate (5 mL) was stirred at 145 °C overnight, then the excess triethyl orthoformate was removed in vacuo. To a solution of the resulted imino ether in dichloromethane (10 mL), trimethylsilyl isocyanate was added (8 mmol, 0.92 g) and stirred at room temperature for two days. Volatiles were removed in vacuo and ether (20 mL) was added to the residue. The resulting precipitate was collected by filter and washed with ether. Mp 270 °C (decomposed). Yield 39% (0.858 g, gray powder). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (d, J=6.3 Hz, 1H), 9.03 (d, J=7.3 Hz, 1H), 8.76 (s, 1H), 7.52 (t, J=6.8 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.5, 154.1, 152.4, 150.5, 130.4, 120.0, 96.7; FABMS (*m*/*e*) 273.9 (MH^+, 66), 248.8 (70), 220.5 (100); HRMS (FAB positive): 273.94719 calcd for $C_7H_5IN_3O$, found 273.9482.

4.4. 2-Methyl-9-iodopyrido[1,2-*a*][1,3,5]triazin-4-one (3b)

This compound was prepared using a similar procedure as used for the synthesis of **3a** with **2** (10 mmol, 2.20 g) and triethyl orthoacetate (5 mL). Yield 48% (1.37 g, white powder). Imino ether: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (br d, J=4.9 Hz, 1H), 8.09 (dd, J=2.0, 7.8 Hz, 1H), 6.71 (dd, J=4.9, 7.8 Hz, 1H), 4.36 (q, J=7.3 Hz, 2H), 1.89 (s, 3H), 1.39 (t, J=7.3 Hz, 3H). Compound **3b**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.91 (dd, J=1.5, 6.8 Hz, 1H), 8.77 (dd, J=1.5, 7.3 Hz, 1H), 7.23 (t, J=6.8 Hz, 1H), 2.44 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.6, 153.2, 152.1, 150.6, 130.1, 119.2, 96.2, 26.5; FABMS (*m*/*e*) 287.8 (M^+, 15), 153.8 (100), 136.0 (66), 107.5 (20), 89.8 (20); HRMS (FAB positive): 287.96284 calcd for $C_8H_7IN_3O$, found 287.9643.

4.5. 2-Amino-9-iodopyrido[1,2-*a*][1,3,5]triazin-4-one (3c)

To a solution of **2** (10 mmol, 2.20 g) in DMF (50 mL) ethoxycarbonyl isothiocyanate (10 mmol, 1.31 g) was added. After stirring at room temperature for 2 h, the reaction mixture was cooled to 0 °C and 0.5 M ammonia in 1,4-dioxane (35 mmol, 70 mL) and mercury(II) chloride (10 mmol, 2.72 g) were added. The mixture was stirred at 0 °C for 15 min, then warmed up to room temperature. Stirring was continued until 4 h had passed, then the mixture was poured into ethyl acetate (75 mL). Precipitation was filtered off using a pad of Celite and the filtrate was evaporated in vacuo. The residue was dissolved in DMF and heated using a microwave at 160 °C for 800 s. The solvent was removed in vacuo and the residue was recrystallized from acetonitrile. Mp 250 °C (decomposed). Yield 58% (1.66 g). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.63 (d, J=6.8 Hz, 1H), 8.47 (d, J=7.3 Hz, 1H), 7.45 (br s, 1H), 7.44 (br s, 1H), 6.77 (t, J=6.8 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.2, 154.0, 150.7, 150.6, 130.0, 114.4, 93.8; HRMS (FAB positive): 288.95809 calcd for $C_7H_6IN_4O$, found 288.9579.

4.6. 2-Dimethylamino-9-iodopyrido[1,2-*a*][1,3,5]triazin-4-one (3d)

To a solution of **2** (10 mmol, 2.20 g) in DMF (50 mL) ethoxycarbonyl isothiocyanate (10 mmol, 1.31 g) was added. After being stirred at room temperature for 2 h, the reaction mixture was cooled down to 0 °C and dimethylamine hydrochloride (25 mmol, 0.815 g), triethylamine (40 mmol, 4.05 g), and mercury(II) chloride (10 mmol, 2.72 g) were added. The mixture was stirred at 0 °C for 15 min, then warmed up to room temperature. Stirring was continued for 4 h and then the mixture was poured into ethyl acetate (75 mL). Precipitation was filtered off using a pad of Celite and the filtrate was evaporated in vacuo. The concentrated residue was dissolved in 1,4-dioxane (50 mL) saturated with hydrogen chloride. After being refluxed for 4 h, saturated potassium bicarbonate solution was poured into the reaction mixture. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layer was dried and concentrated. The product was purified by flash column chromatography (dichloromethane–ethyl acetate=4:1, R_f=0.35). Yield 85% (2.67 g, white powder). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.62 (dd, J=1.0, 6.8 Hz, 1H), 8.47 (dd, J=1.5, 7.3 Hz, 1H), 6.78 (t, J=6.8 Hz, 1H), 2.95 (s, 3H), 3.03 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.4, 153.1, 150.5, 150.2, 129.9, 114.5. 94.6, 36.4, 36.0; FABMS (*m*/*e*) 316.8 (MH^+, 56), 153.8 (100), 136.0 (70), 85.9 (68), 306.9 (25); HRMS (FAB positive): 316.98939 calcd for $C_9H_{10}IN_4O$, found 316.9893.

4.7. 2-Isobutyrylamino-9-iodopyrido[1,2-*a*][1,3,5]triazin-4-one (3e)

To a solution of **3c** (0.5 mmol, 144 mg) in pyridine (2.8 mL) isobutyryl chloride (0.75 mmol, 79 μL) was added dropwise. After being stirred at 115 °C for 2 h, the reaction mixture was concentrated in vacuo and then purified by flash column chromatography (*n*-hexane–ethyl acetate=2:1). Yield 98% (175 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.42 (s, 1H), 8.84 (d, J=7.8 Hz, 1H), 8.70 (d, J=7.3 Hz, 1H), 7.10 (t, J=7.3 Hz, 1H), 3.51–3.42 (m, 1H), 1.11 (d, J=6.8 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.8, 160.8, 154.5, 152.0, 151.1, 130.3, 117.7, 95.4, 34.3, 19.2; HRMS (FAB positive): 358.99995 calcd for $C_{11}H_{12}IN_4O_2$, found 359.0016.

4.8. General procedure of Heck-type palladium-catalyzed reaction

A reaction tube containing a mixture of aryl iodide (1 equiv), tris(dibenzylidene)acetone dipalladium(0) chloroform adduct (5 mol %), tri(*tert*-butyl)phosphine (20 mol %), furanoid glycal **10** (1 equiv), and diisopropylethylamine (4.5 equiv) in DMF (5 mL/mmol) was sealed. The mixture was heated using a microwave at 160 °C for 10 min. The product was isolated by flash column chromatography.

4.8.1. 2*R*,5*R*-2-(2-Aminopyridin-3-yl)-4-(*tert*-butyldimethylsiloxy)-5-(*tert*-butyldimethylsiloxymethyl)-2,5-dihydrofuran (4). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (dd, J=1.5, 4.9 Hz, 1H), 7.30 (dd, J=1.5, 7.3 Hz, 1H), 6.55 (dd, J=5.4, 7.3 Hz, 1H), 5.63 (d, J=3.4 Hz, 1H), 5.32 (br s, 2H), 4.86 (s, 1H), 4.53 (dd, J=2.0, 2.4 Hz, 1H), 3.87 (dd, J=2.9, 11.7 Hz, 1H), 3.80 (dd, J=2.9, 11.7 Hz, 1H), 0.95 (s, 9H), 0.86 (s, 9H), 0.24 (s, 3H), 0.23 (s, 3H), 0.01 (s, 3H), 0.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.7, 152.0, 147.5, 136.9, 119.2, 112.9, 99.9, 85.3, 83.2, 63.2, 26.0, 25.6, 18.7, 18.1, −4.9, −5.4, −5.5; FABMS (*m*/*e*) 437.0 (MH^+, 19), 287.0 (10), 246.9 (7), 74.4 (100); HRMS (FAB positive): 437.26502 calcd for $C_{22}H_{41}N_2O_3Si_2$, found 437.2674.

4.8.2. 2-Methyl-9-[2*R*,5*R*-5-(*tert*-butyldimethylsiloxymethyl)-4-oxo-tetrahydrofuran-2-yl]-pyrido[1,2-*a*][1,3,5]triazin-4-one (5b). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.99 (dd, J=1.0, 6.8 Hz, 1H), 8.48 (dd, J=1.5, 7.3 Hz, 1H), 7.35 (t, J=7.3 Hz, 1H), 5.78 (dd, J=6.3, 10.7 Hz, 1H), 4.15 (t, J=2.0 Hz, 1H), 4.04 (dd, J=2.0, 11.2 Hz, 1H), 3.99 (dd, J=2.9, 11.2 Hz, 1H), 3.31 (dd, J=6.3, 18.1 Hz, 1H), 2.54 (s, 3H), 2.19 (dd, J=10.7, 18.1 Hz, 1H), 0.84 (s, 9H), 0.09 (s, 3H), 0.05 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 212.6, 175.0, 152.2, 150.2, 137.2, 136.8, 128.2, 117.1, 82.1, 71.9, 62.4, 44.5, 26.6, 25.5, 18.0, −5.7, −5.9; HRMS (FAB positive): 390.18436 calcd for $C_{19}H_{28}N_3O_4Si$, found 390.1867.

4.8.3. 2-Dimethylamino-9-[2*R*,5*R*-5-(*tert*-butyldimethylsiloxymethyl)-4-oxo-tetrahydrofuran-2-yl]-pyrido[1,2-*a*][1,3,5]triazin-4-one (5d). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (dd, J=1.5, 7.3 Hz, 1H), 8.15 (d, J=6.8 Hz, 1H), 7.35 (t, J=6.8 Hz, 1H), 5.57 (dd, J=5.9, 10.2 Hz, 1H), 4.12 (t, J=2.4 Hz, 1H), 4.02 (dd, J=2.4, 11.2 Hz, 1H), 3.97 (dd, J=2.4, 11.2 Hz, 1H), 3.27–3.16 (m, 1H), 3.24 (s, 3H), 3.21 (s, 3H), 2.19 (dd, J=10.2, 18.6 Hz, 1H), 0.83 (s, 9H), 0.08 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 213.7, 161.4, 152.7, 150.8, 135.6, 134.8, 128.6, 112.4, 82.3, 72.3, 62.6, 44.2, 37.05, 36.95, 25.7, 18.2, −5.5, −5.7; FABMS (*m*/*e*) 419.0 (MH^+, 57), 74.2 (100), 216.7 (19); HRMS (FAB positive): 419.21091 calcd for $C_{20}H_{31}N_4O_4Si$, found 419.2128.

4.8.4. 9-[2*R*,5*R*-4-(*tert*-butyldimethylsiloxy)-5-(*tert*-butyldimethylsiloxymethyl)-2,5-dihydrofuran-2-yl]-pyrido[1,2-*a*][1,3,5]triazin-4-one (6a). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.01 (dd, J=1.5, 6.8 Hz, 1H), 8.71 (d, J=7.3 Hz, 1H), 8.60 (s, 1H), 7.38 (t, J=6.8 Hz, 1H), 6.34 (s, 1H), 5.04 (s, 1H), 4.66 (dd, J=2.5, 4.5 Hz, 1H), 3.97 (dd, J=2.4, 11.7 Hz, 1H), 3.83 (dd, J=2.4, 11.2 Hz, 1H), 0.94 (s, 9H), 0.87 (s, 9H), 0.24 (s, 3H), 0.17 (s, 3H), 0.09 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.8, 153.0, 150.6, 150.5, 139.7, 139.1, 128.1, 118.5, 100.3, 84.3, 79.0, 62.9, 25.9, 25.5, 18.4, 17.9, −4.9, −5.2, −5.4; HRMS (FAB positive): 490.25519 calcd for $C_{24}H_{40}N_3O_4Si_2$, found 490.2556.

4.8.5. 2-Amino-9-[2*R*,5*R*-4-(*tert*-butyldimethylsiloxy)-5-(*tert*-butyldimethylsiloxymethyl)-2,5-dihydrofuran-2-yl]-pyrido[1,2-*a*][1,3,5]triazin-4-one (6c). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (dd, J=1.5, 6.8 Hz, 1H), 8.11 (d, J=6.8 Hz, 1H), 7.32 (br s, 1H), 7.31 (br s, 1H), 6.99 (t, J=7.3 Hz, 1H), 6.04 (d, J=3.9 Hz, 1H), 5.09 (s, 1H), 4.56 (s, 1H), 3.84 (dd, J=2.0, 11.2 Hz, 1H), 3.72 (dd, J=2.9, 11.2 Hz, 1H), 0.91 (s, 9H), 0.83 (s, 9H), 0.22 (s, 3H), 0.16 (s, 3H), 0.04 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.9, 153.8, 151.2, 150.3, 138.0, 136.2, 128.1, 113.4, 100.4, 84.1, 78.8, 63.2, 25.8, 25.4, 18.4, 17.9, −4.9, −5.2, −5.4; HRMS (FAB positive): 505.26609 calcd for $C_{24}H_{41}N_4O_4Si_2$, found 505.2672.

4.8.6. 2-Dimethylamino-9-[2*R*,5*R*-4-(*tert*-butyldimethylsiloxy)-5-(*tert*-butyldimethylsiloxymethyl)-2,5-dihydrofuran-2-yl]-pyrido[1,2-*a*][1,3,5]triazin-4-one (6d). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, J=6.8 Hz, 1H), 8.20 (d, J=6.8 Hz, 1H), 6.82 (t, J=6.8 Hz, 1H), 6.12 (s, 1H), 5.11 (s, 1H), 4.64 (br s, 1H), 3.95 (dd, J=2.4, 11.2 Hz, 1H), 3.79 (dd, J=3.4, 11.7 Hz, 1H), 3.25 (s, 3H), 3.24 (s, 3H), 0.94 (s, 9H), 0.87 (s, 9H), 0.22 (s, 3H), 0.16 (s, 3H), 0.08 (s, 3H), 0.03 (s, 3H); FABMS (*m*/*e*) 532.8 (MH^+, 51), 74.2 (100), 216.7 (3), 387.1 (3); HRMS (FAB positive): 533.29739 calcd for $C_{26}H_{45}N_4O_4Si_2$, found 533.2984.

4.8.7. 2-Isobutyrylamino-9-[2*R*,5*R*-4-(*tert*-butyldimethylsiloxy)-5-(*tert*-butyldimethylsiloxymethyl)-2,5-dihydrofuran-2-yl]-pyrido[1,2-*a*][1,3,5]triazin-4-one (6e). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (d, J=6.8 Hz, 1H), 8.52 (d, J=7.2 Hz, 1H), 7.70 (s, 1H), 7.10 (t, J=7.2 Hz, 1H), 6.22 (s, 1H), 5.11 (s, 1H), 4.66 (s, 1H), 3.97–3.79 (m, 2H), 3.61–3.55 (m, 1H), 1.27 (d, J=6.8 Hz, 6H), 0.93 (s, 9H), 0.89 (s, 9H), 0.24 (s, 3H), 0.17 (s, 3H), 0.09 (s, 3H), 0.03 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.7, 159.5, 153.2, 150.9, 150.5, 139.8, 138.2, 128.3, 116.6, 100.3, 84.3, 79.2, 63.0, 35.1, 25.9, 25.5, 19.1, 19.0, 18.4, 18.0, −4.9, −5.2, −5.4; FABMS (*m*/*e*) 574.9 (M^+, 93), 429.0 (7), 74.2 (100); HRMS (FAB positive): 575.30795 calcd for $C_{28}H_{47}N_4O_5Si_2$, found 575.3086.

4.9. General procedure of the deprotection of Heck reaction adduct 6

A mixture of **6** (1 equiv), 75% tetrabutylammonium fluoride (4 equiv), acetic acid (8 equiv), and THF (10 mL/mmol) was stirred at room temperature overnight. After concentration in vacuo, the product was purified by flash column chromatography.

4.9.1. 9-(2*R*,5*R*-5-Hydroxymethyl-4-oxo-tetrahydrofuran-2-yl)-pyrido[1,2-*a*][1,3,5]triazin-4-one (7a). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.96 (d, J=6.8 Hz, 1H), 8.61 (d, J=7.3 Hz, 1H), 8.50 (s, 1H), 7.67 (t, J=6.8 Hz, 1H), 5.77 (dd, J=6.3, 9.8 Hz, 1H), 5.08 (t, J=5.4 Hz, 1H), 4.14 (s, 1H), 3.82–3.65 (m, 2H), 3.06 (dd, J=6.3, 18.1 Hz, 1H), 2.28 (dd, J=9.8, 18.1 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 213.5, 163.4, 152.8, 150.4, 138.4, 136.6, 128.7, 119.0, 82.3, 71.4, 60.4, 44.0; FABMS (*m*/*e*) 261.1 (M^+, 7), 236.8 (7), 218.8 (10), 190.7 (15), 58.6 (100); HRMS (FAB positive): 262.08223 calcd for $C_{12}H_{12}N_3O_4$, found 262.0820.

4.9.2. 2-Methyl-9-(2*R*,5*R*-5-hydroxymethyl-4-oxo-tetrahydrofuran-2-yl)-pyrido[1,2-*a*][1,3,5]triazin-4-one (7b). ^{1}H NMR (400 MHz, CD_3OD) δ 8.89 (dd, J=1.5, 6.8 Hz, 1H), 8.51 (dd, J=1.0, 6.8 Hz, 1H), 7.47 (t, J=7.3 Hz, 1H), 5.69 (dd, J=6.4, 10.3 Hz, 1H), 4.06 (t, J=3.4 Hz, 1H),

3.86 (dd, J=2.9, 12.2 Hz, 1H), 3.80 (dd, J=3.4, 12.2 Hz, 1H), 3.15 (dd, J=6.3, 18.1 Hz, 1H), 2.22 (dd, J=10.2, 18.1 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 213.6, 173.5, 152.2, 149.9, 138.1, 135.9, 128.4, 118.0, 82.3, 71.6, 60.5, 44.0, 26.4; HRMS (FAB positive): 276.09788 calcd for $C_{13}H_{14}N_3O_4$, found 276.0979.

4.9.3. 2-Amino-9-(2*R*,5*R*-5-hydroxymethyl-4-oxo-tetrahydrofuran-2-yl)-pyrido[1,2-*a*][1,3,5]triazin-4-one (7c). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (dd, J=1.0, 6.8 Hz, 1H), 8.19 (d, J=6.8 Hz, 1H), 7.34 (br s, 1H), 7.20 (br s, 1H), 7.08 (t, J=6.8 Hz, 1H), 5.44 (dd, J=6.3, 10.7 Hz, 1H), 5.03 (br s, 1H), 4.10 (t, J=2.9 Hz, 1H), 3.78–3.62 (m, 2H), 3.12 (dd, J=5.9, 18.1 Hz, 1H), 2.25 (dd, J=10.7, 18.1 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 214.5, 163.8, 153.5, 150.6, 136.4, 134.0, 128.3, 113.2, 82.5, 71.8, 60.6, 44.1; HRMS (FAB positive): 277.09313 calcd for $C_{12}H_{13}N_4O_4$, found 277.0937.

4.9.4. 2-Dimethylamino-9-(2*R*,5*R*-5-hydroxymethyl-4-oxo-tetrahydrofuran-2-yl)-pyrido[1,2-*a*][1,3,5]triazin-4-one (7d). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (d, J=6.8 Hz, 1H), 8.22 (d, J=6.8 Hz, 1H), 7.08 (t, J=6.8 Hz, 1H), 5.57 (dd, J=6.4, 10.3 Hz, 1H), 5.01 (t, J=6.4 Hz, 1H), 4.11 (t, J=2.9 Hz, 1H), 3.76 (ddd, J=2.9, 5.4, 12.2 Hz, 1H), 3.68 (ddd, J=3.42, 6.4, 12.2 Hz, 1H), 3.19 (s, 3H), 3.119 (s, 3H), 3.117 (dd, J=7.3, 18.1 Hz, 1H), 2.22 (dd, J=10.2, 18.1 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 214.1, 160.9, 152.4, 149.7, 136.0, 134.4, 128.0, 113.1, 82.2, 71.6, 60.4, 43.5, 36.5, 36.1; FABMS (*m*/*e*) 304.9 (MH$^+$, 22), 242.0 (100), 153.8 (46), 136.0 (31), 183.8 (8); HRMS (FAB positive): 305.12443 calcd for $C_{14}H_{17}N_4O_4$, found 305.1260.

4.9.5. 2-Isobutyrylamino-9-(2*R*,5*R*-5-hydroxymethyl-4-oxo-tetrahydrofuran-2-yl)-pyrido[1,2-*a*][1,3,5]triazin-4-one (7e). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95 (d, J=7.2 Hz, 1H), 8.63 (br s, 1H), 8.36 (d, J=7.2 Hz, 1H), 7.30 (t, J=6.8 Hz, 1H), 5.69 (dd, J=6.4, 10.7 Hz, 1H), 4.16 (t, J=3.4 Hz, 1H), 4.04 (d, J=3.4 Hz, 2H), 3.59 (dd, J=6.3, 18.5 Hz, 1H), 3.02–2.90 (m, 1H), 2.37 (dd, J=10.7, 18.5 Hz, 1H), 1.26 (d, J=6.8 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 214.2, 168.9, 145.3, 143.5, 129.5, 128.0, 120.2, 108.3, 105.0, 74.4, 64.1, 52.3, 30.3, 27.4, 10.2, 10.1; FABMS (*m*/*e*) 346.9 (MH$^+$, 6), 306.9 (26), 288.9 (14), 242.0 (9), 153.8 (100), 136.0 (63); HRMS (FAB positive): 347.13500 calcd for $C_{16}H_{19}N_4O_5$, found 347.1365.

4.10. General procedure of the reduction of 3′-keto-nucleoside 7

To a stirring solution of **7** (1 equiv) in acetic acid (20 mL/mmol) and acetonitrile (60 mL/mmol), sodium tri(acetoxy)-borohydride (1.1 equiv) was slowly added at −40 °C. Stirring was continued for 1 h and then the mixture was allowed to warm to room temperature. The solvent was removed in vacuo and the product was purified by flash column chromatography.

4.10.1. 9-(2′-Deoxy-β-D-ribofuranosyl)-pyrido[1,2-*a*][1,3,5]triazin-4-one (8a). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (dd, J=1.5, 6.8 Hz, 1H), 8.53 (dd, J=1.0, 7.3 Hz, 1H), 8.49 (s, 1H), 7.60 (t, J=7.3 Hz, 1H), 5.71 (dd, J=5.9, 9.8 Hz, 1H), 4.33 (t, J=2.9 Hz, 1H), 4.02 (dt, J=2.9, 4.9 Hz, 1H), 3.77–3.66 (m, 2H), 2.64 (ddd, J=2.0, 5.9, 13.2 Hz, 1H), 1.85 (ddd, J=5.9, 9.8, 13.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.4, 154.5, 152.3, 140.1, 139.9, 129.7, 120.4, 89.1, 76.5, 74.2, 63.8, 43.4.

4.10.2. 2-Methyl-9-(2′-deoxy-β-D-ribofuranosyl)-pyrido[1,2-*a*][1,3,5]triazin-4-one (8b). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (d, J=6.8 Hz, 1H), 8.35 (d, J=7.3 Hz, 1H), 7.51 (t, J=7.3 Hz, 1H), 5.54 (dd, J=6.3, 9.7 Hz, 1H), 5.15 (d, J=3.9 Hz, 1H), 4.84 (t, J=5.4 Hz, 1H), 4.20 (br s, 1H), 3.89–3.85 (m, 1H), 3.57–3.44 (m, 2H), 2.42 (s, 3H), 1.76–1.68 (m, 1H), 1.61–1.52 (m, 1H); FABMS (*m*/*e*) 278.0 (MH$^+$, 10), 242.0 (100), 85.9 (32); HRMS (FAB positive): 278.11353 calcd for $C_{13}H_{16}N_3O_4$, found 278.1148.

4.10.3. 2-Amino-9-(2′-deoxy-β-D-ribofuranosyl)-pyrido[1,2-*a*][1,3,5]triazin-4-one (8c). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (d, J=6.8 Hz, 1H), 8.02 (d, J=6.8 Hz, 1H), 7.27 (br s, 2H), 7.02 (t, J=6.8 Hz, 1H), 5.37 (dd, J=5.9, 9.3 Hz, 1H), 5.10 (d, J=3.9 Hz, 1H), 4.81 (t, J=5.9 Hz, 1H), 4.10 (br s, 1H), 3.82 (br s, 1H), 3.53–3.43 (m, 2H), 2.42 (dd, J=4.9, 11.7 Hz, 1H), 1.67 (ddd, J=5.9, 9.8, 12.7 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.6 153.2, 150.2, 135.8, 135.7, 127.6, 112.8, 87.4, 74.0, 72.0, 62.1, 41.5; FABMS (*m*/*e*) 279.0 (MH$^+$, 4), 153.8 (100), 136.0 (64), 253.0 (16); HRMS (FAB positive): 279.10878 calcd for $C_{12}H_{15}N_4O_4$, found 279.1167.

4.10.4. 2-Dimethylamino-9-(2′-deoxy-β-D-ribofuranosyl)-pyrido[1,2-*a*][1,3,5]triazin-4-one (8d). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.54 (d, J=6.8 Hz, 1H), 8.01 (d, J=7.3 Hz, 1H), 7.02 (t, J=6.8 Hz, 1H), 5.35 (t, J=6.8 Hz, 1H), 5.09 (t, J=2.0 Hz, 1H), 4.78 (t, J=5.9 Hz, 1H), 4.15 (br s, 1H), 3.84 (br s, 1H), 3.51–3.44 (m, 2H), 3.20 (s, 3H), 3.12 (s, 3H), 2.54–2.44 (m, 1H), 1.70–1.62 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.9 152.2, 147.0, 136.1, 135.4, 127.4, 113.1, 87.3, 74.4, 71.9, 62.1, 41.2, 36.4, 36.1; FABMS (*m*/*e*) 306.9 (MH$^+$, 46), 153.8 (100), 136.0 (67), 85.9 (48), 288.9 (15), 237.8 (11); HRMS (FAB positive): 307.14008 calcd for $C_{14}H_{19}N_4O_4$, found 307.1409.

4.10.5. 2-Isobutyrylamino-9-(2′-deoxy-β-D-ribofuranosyl)-pyrido[1,2-*a*][1,3,5]triazin-4-one (8e). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.44 (s, 1H), 8.79 (d, J=7.3 Hz, 1H), 8.29 (d, J=7.3 Hz, 1H), 7.39 (t, J=7.3 Hz, 1H), 5.48 (dd, J=6.4, 9.3 Hz, 1H), 5.13 (d, J=3.9 Hz, 1H), 4.83 (br s, 1H), 4.19 (br s, 1H), 3.87 (d, J=2.9 Hz, 1H), 3.55–3.45 (m, 2H), 3.18–3.10 (m, 1H), 2.65–2.58 (m, 1H), 1.77–1.69 (m, 1H), 1.11 (d, J=6.3 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.2, 159.8, 152.9, 150.6, 137.7, 137.3, 128.0, 116.4, 87.5, 74.3, 71.9, 62.1, 41.5, 34.4, 19.1, 19.0; FABMS (*m*/*e*) 348.9 (MH$^+$, 82), 242.0 (100), 186.0 (85), 85.9 (97); HRMS (FAB positive): 349.15065 calcd for $C_{16}H_{21}N_4O_5$, found 349.1526.

4.11. General procedure of tritylation of artificial nucleoside 8

A solution of **8** (1 equiv), diisopropylethylamine (3 equiv), and 4,4′-dimethoxytrityl chloride (1.5 equiv) in pyridine (10 mL/mmol) was stirred at room temperature for 1 h.

The mixture was concentrated in vacuo and the residue was subjected to purification by flash column chromatography.

4.11.1. 2-Methyl-9-[2′-deoxy-5′-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]-pyrido[1,2-*a*][1,3,5]triazin-4-one (9b). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (d, J=6.8 Hz, 1H), 8.25 (d, J=6.8 Hz, 1H), 7.46–7.17 (m, 10H), 6.86–6.78 (m, 4H), 5.71 (dd, J=6.3, 8.9 Hz, 1H), 4.44 (dt, J=2.9, 5.8 Hz, 1H), 4.17 (dd, J=4.9, 8.3 Hz, 1H), 3.79 (s, 6H), 3.40 (dd, J=4.9, 10.3 Hz, 1H), 3.36 (dd, J=4.9, 10.3 Hz, 1H), 2.74 (ddd, J=2.4, 6.3, 13.2 Hz, 1H), 2.55 (s, 3H), 1.91 (ddd, J=6.3, 9.3, 15.6 Hz, 1H); FABMS (*m*/*e*) 579.9 (M^+, 9), 441.0 (40), 303.0 (40), 146.9 (23), 58.6 (100); HRMS (FAB positive): 580.24421 calcd for $C_{34}H_{34}N_3O_6$, found 580.2450.

4.11.2. 2-Dimethylamino-9-[2′-deoxy-5′-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]-pyrido[1,2-*a*][1,3,5]triazin-4-one (9d). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (dd, J=1.5, 6.8 Hz, 1H), 7.91 (d, J=6.8 Hz, 1H), 7.47–7.17 (m, 10H), 6.87–6.81 (m, 4H), 6.75 (t, J=6.8 Hz, 1H), 5.52 (t, J=7.8 Hz, 1H), 4.37 (br s, 1H), 4.12–4.09 (m, 1H), 3.79 (s, 6H), 3.40 (dd, J=4.9, 10.2 Hz, 1H), 3.33 (dd, J=4.9, 10.2 Hz, 1H), 3.24 (s, 6H), 2.65 (ddd, J=3.4, 6.8, 13.2 Hz, 1H), 1.91 (ddd, J=6.3, 8.8, 15.1 Hz, 1H); FABMS (*m*/*e*) 609.0 (MH^+, 12), 58.6 (100), 302.9 (58), 441.0 (35); HRMS (FAB positive): 609.27076 calcd for $C_{35}H_{37}N_4O_6$, found 609.2706.

4.11.3. 2-Isobutyrylamino-9-[2′-deoxy-5′-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]-pyrido[1,2-*a*][1,3,5]triazin-4-one (9e). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.46 (s, 1H), 8.76 (d, J=6.8 Hz, 1H), 8.11 (d, J=6.8 Hz, 1H), 7.44–7.18 (m, 10H), 6.93–6.85 (m, 4H), 5.48 (t, J=7.8 Hz, 1H), 5.20 (d, J=4.4 Hz, 1H), 4.14 (br s, 1H), 4.04–3.96 (m, 1H), 3.74 (s, 6H), 3.20–3.15 (m, 3H), 2.68–2.61 (m, 1H), 1.82–1.73 (m, 1H), 1.10 (d, J=6.8 Hz, 6H); HRMS (FAB positive): 651.28133 calcd for $C_{37}H_{39}N_4O_7$, found 651.2799.

4.12. General procedure of the preparation of phosphoramidite 1

Compound **9** (1 equiv) was dissolved in anhydrous CH_2Cl_2 (10 mL/mmol). Then, diisopropylethylamine (6 equiv), 1*H*-tetrazole (6 equiv), and 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite (1.5 equiv) were added and stirred at room temperature for 3 h under nitrogen atmosphere. The reaction mixture was purified by silica gel column chromatography with CH_2Cl_2–THF as eluent to give phosphoramidite.

4.12.1. 2-Methyl-9-[3′-(2-cyanoethoxydiisopropylamino)phosphinyl-2′-deoxy-5′-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]-pyrido[1,2-*a*][1,3,5]triazin-4-one (1b). ^{31}P NMR (160 MHz, $CDCl_3$) δ 148.8, 148.0.

4.12.2. 2-Dimethylamino-9-[3′-(2-cyanoethoxydiisopropylamino)phosphinyl-2′-deoxy-5′-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]-pyrido[1,2-*a*][1,3,5]triazin-4-one (1d). ^{31}P NMR (160 MHz, $CDCl_3$) δ 148.4, 147.7.

4.12.3. 2-Isobutyrylamino-9-[3′-(2-cyanoethoxydiisopropylamino)phosphinyl-2′-deoxy-5′-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]-pyrido[1,2-*a*][1,3,5]triazin-4-one (1e). ^{31}P NMR (160 MHz, DMSO-d_6) δ 147.56, 146.98. FABMS (*m*/*e*) 851.3 (M^+, 12), 302.1 (97), 363.8 (15), 459.8 (6).

4.13. Synthesis of oligomer

All oligonucleotides were synthesized at 0.2 mmol scale on a Millipore Expedite 8909 DNA synthesizer using conventional β-cyanoethyl phosphoramidite chemistry. The standard and the modified bases were dissolved in anhydrous acetonitrile (0.1 M final concentration). All oligomers were synthesized 'trityl on'. After the synthesis, the solid supports were treated overnight at 55 °C with concentrated ammonia (1 mL). The crude tritylated oligonucleotide was purified and deprotected by reverse-phase cartridge (Poly-Pak). Further purification was carried out using ion-exchange column (TOSOH, TSK-gel DNA-NPR) with buffer A (Tris–HCl 0.1 M, pH 9.0) and buffer B (Tris–HCl 0.1 M, sodium chloride 1.0 M, pH 9.0). A linear gradient of 35–50% buffer B over 20 min at a flow of 0.75 mL/min was used. The oligonucleotides were desalted and dried on a Speed-Vac evaporator to yield colorless solids. ESI-TOF mass spectra are used for characterization.

5′-GGTAAC-**8d**-ATGCG-3′ (E.M. 3748.4): ESI-mass: 1907.5 (2−, 3Na$^+$), 1917.5 (2−, 4Na$^+$), 1929.0 (2−, 5Na$^+$), 1938.9 (2−, 6Na$^+$).

5′-CGCAT-**8d** -GTTACC-3′ (E.M. 3659.40): ESI-mass: 1219.1 (3−), 1226.4 (3−, Na$^+$), 1233.8 (3−, 2Na$^+$), 1240.9 (3−, 3Na$^+$), 1248.4 (3−, 4Na$^+$), 1255.5 (3−, 5Na$^+$), 1263.1 (3−, 6Na$^+$), 1270.27 (3−, 7Na$^+$).

4.14. T_m analysis

Purified oligonucleotides, 0.5 mmol of each, were dissolved in 3.0 mL of the buffer (10 mM sodium phosphate, 100 mM sodium chloride, and 0.1 mM EDTA, pH 7.0). Samples were kept at least 2 min at 10 °C and were then heated from 1070 °C to 70 °C at a rate of 0.5 °C/min. The absorbance at 260 nm was measured every 10 s.

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References and notes

1. Ulrich, H.; Trujillo, C. A.; Nery, A. A.; Alves, J. M.; Majumder, P.; Resende, R. R.; Martins, A. H. *Comb. Chem. High Throughput Screen.* **2006**, *9*, 619–632; Hamula, C. L. A.; Guthrie, J. W.; Zhang, H.; Li, X.-F.; Le, X. C. *Trends Anal. Chem.* **2006**, *25*, 681–691; Jaschke, A.; Keiper, S. *Highlights Bioorg. Chem.* **2004**, 433–435.
2. Yang, Z.; Hutter, D.; Sheng, P.; Sismour, A. M.; Benner, S. A. *Nucleic Acids Res.* **2006**, *34*, 6095–6101; Leconte, A. M.;

Matsuda, S.; Hwang, G. T.; Romesberg, F. E. *Angew. Chem., Int. Ed.* **2006**, *45*, 4326–4329; Hirao, I.; Kimoto, M.; Mitsui, T.; Fujiwara, T.; Kawai, R.; Sato, A.; Harada, Y.; Yokoyama, S. *Nat. Methods* **2006**, *3*, 729–735; Rappaport, H. P. *Biochem. J.* **2004**, *381*, 709–717; Lee, A. H. F.; Delaney, J. C.; Henderson, P. T.; Helquist, S. A.; Morales, J. C.; Essigmann, J. M.; Kool, E. T. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 4469–4473.

3. Kato, T.; Masuda, S. *Chem. Pharm. Bull.* **1975**, *23*, 2251–2256.
4. Genin, M. J.; Allwine, D. A.; Anderson, D. A.; Berbachyn, M. R.; Emmert, D. E.; Garmon, S. A.; Gerber, D. R.; Grega, K. C.; Hester, J. B.; Hutchinson, D. K.; Morris, J.; Reischer, R. J.; Ford, C. W.; Zurenko, G. E.; Hamel, J. C.; Schaadt, R. D.; Stapert, D.; Yagi, B. H. *J. Med. Chem.* **2000**, *43*, 953–970; Baraldi, P. G.; Fruttarolo, F.; Tabrizi, M. A.; Preti, D.; Romagnoli, R.; El-Kashef, H.; Moorman, A.; Varani, K.; Gessi, S.; Merighi, S.; Borea, P. A. *J. Med. Chem.* **2003**, *46*, 1229–1241.
5. Cameron, M. A.; Cush, S. B.; Hammer, R. P. *J. Org. Chem.* **1997**, *62*, 9065–9069.
6. Zhang, H.-C.; Daves, G. D., Jr. *J. Org. Chem.* **1992**, *57*, 4690–4696.
7. Seela, F.; Melenewski, A. *Eur. J. Org. Chem.* **1999**, 485–496.
8. Klapars, A.; Buchwald, S. L. *J. Am. Chem. Soc.* **2002**, *124*, 14844–14845.