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Synthesis of 5-acetyl-2-aminopyrrole *C*-deoxyribonucleoside

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Abstract—A novel *C*-deoxyribonucleoside bearing 2-aminopyrrole was synthesized. The *C*-glycosidation between deoxyribose and pyrrole ring was carried out using palladium-catalyzed Heck coupling in the presence of excess amount of lithium chloride as an additive. The synthesized aminopyrrole nucleoside is expected to become a promising counterpart of the artificial nucleoside pair.

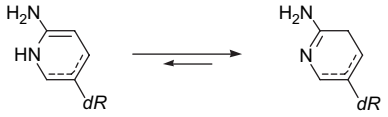
1. Introduction

DNA that consists of the four bases A, G, C, and T is one of the most important biomolecules by means of coding indispensable information for life. The three bases of mRNA transcribed from DNA, codon, correspond to 20 kinds of amino acids respectively, and translated to protein. Thus, if an artificial nucleobase pair could be added to the natural base pair system, the number of codons would be dramatically increased. In the past two decades, some groups reported the trial of synthesis of enzymatically replicable artificial base pairs for a functionalized protein that includes unnatural amino acids by such an approach.[1]

The artificial base pairs that have been reported so far can be divided roughly into hydrogen bond type and hydrophobic interaction type according to how to form the base pair. At present, there is a report about highly selective PCR replication including artificial base pairs using the hydrophobic interaction type base pair.[1e] However, these bases can hardly be used in a codon because the bonding energy is quite low.

Benner et al. vigorously studied the syntheses of several artificial nucleosides that had different hydrogen bonding patterns from natural bases.[1a–c] They employed artificial bases that had triple hydrogen bonds like the natural G/C base pair. When isoG/isoC base pair was used for the experiment of incorporation by DNA polymerase, moderate selectivity was achieved in their replication.[1b]

If a nucleoside pair is formed by using two hydrogen bonds like the natural A/T base pair, four hydrogen bonding patterns can be depicted as shown in Figure 1 because hydrogen bond has the direction. However, it is possible that pyrimidine-type bases shown in Figure 1**A**–**C** form pairs with natural purine bases. Although type **D** that has *gem*-diaminoethylene structure is unstable, and tautomerization occurs easily to take an undesired hydrogen bonding pattern as shown in Eq. 1,[2] this six-membered base is promising because it is expected to form a specific base pair with unnatural purine-type base.



(1)

On the other hand, we recently reported the synthesis of imidazo[1,2-*a*][1,3,5]triazin-4-one deoxyribonucleoside (**Im**) as a purine-type nucleoside that only has solely lone-pair

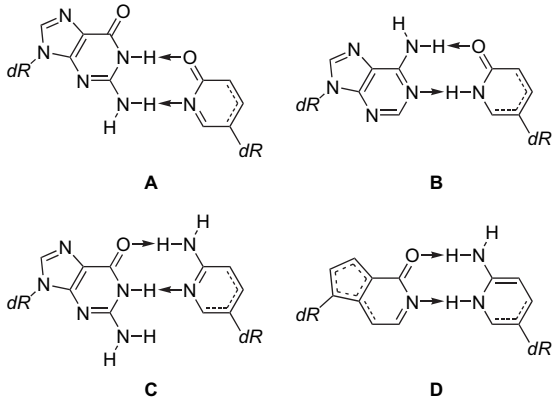


Figure 1. Base pairing of double hydrogen bonded deoxyribonucleoside.

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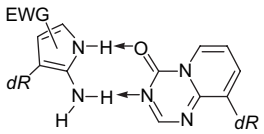


Figure 2. Base pairing of 2-aminopyrrole and pyrido[1,2-*a*][1,3,5]triazin-4-one.

donor site located in one molecule.[3a] Furthermore, we described the synthesis of pyrido[1,2-*a*][1,3,5]triazin-4-one *C*-deoxyribonucleoside (**Pt**) and a DNA oligomer including this nucleoside, and revealed that the derivative of this nucleoside does not form base pairs with all four natural bases efficiently.[3b] When introducing the fused six/six-membered ring into the natural DNA strand, it is preferable to employ the five-membered ring to fit the distance between the anomer carbons of the complementary strand to the natural nucleoside (Fig. 2). Naturally, a triple hydrogen bond is stronger than a double hydrogen bond. However, to consider the secondary electrostatic interaction, nucleoside **1** is expected to form a stronger bond than an antiparallel hydrogen bond such as A/T base pair.[4]

It is difficult to design an appropriate pyrimidine-type base that has only lone-pair acceptor sites satisfying the restriction of the nucleobase such as a planarity, not markedly large size. Thus, we planned to synthesize an artificial nucleoside bearing 2-aminopyrrole as shown in Figure 2. Although 2-aminopyrrole is frequently seen in the field of computational chemistry, this compound is unstable in air. There is only one report available where 2-aminopyrrole was confirmed in the NMR tube.[5] It is necessary to introduce an electron withdrawing group in order to stabilize the *gem*-diaminoethylene structure of 2-aminopyrrole. We used 2-acetylpyrrole as a readily available starting pyrrole having an electron withdrawing group, and report herein the synthesis of novel *C*-deoxyribonucleoside, 5-acetyl-2-aminopyrrole *C*-deoxyribonucleoside **1** (Fig. 3) as a promising counterpart of the **Im** or **Pt**.

The nucleoside **1** may be usable for cDNA synthesis containing modified RNA such as trimethylguanosine as shown in Figure 4. We have previously prepared and sequenced full-length cDNA from several organisms so far.[6] However, we may not have achieved the sequence of all cDNAs, because mRNA contains many modified RNA residues. If there is no

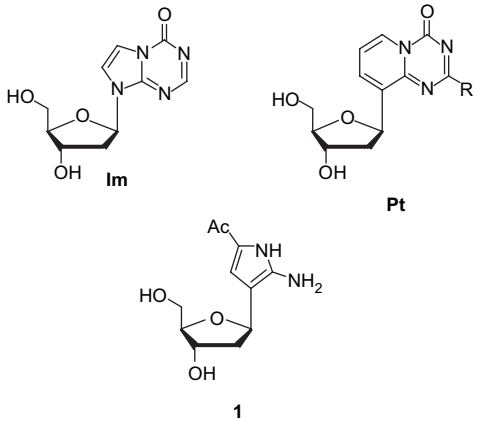


Figure 3. Artificial nucleoside.

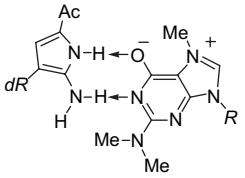
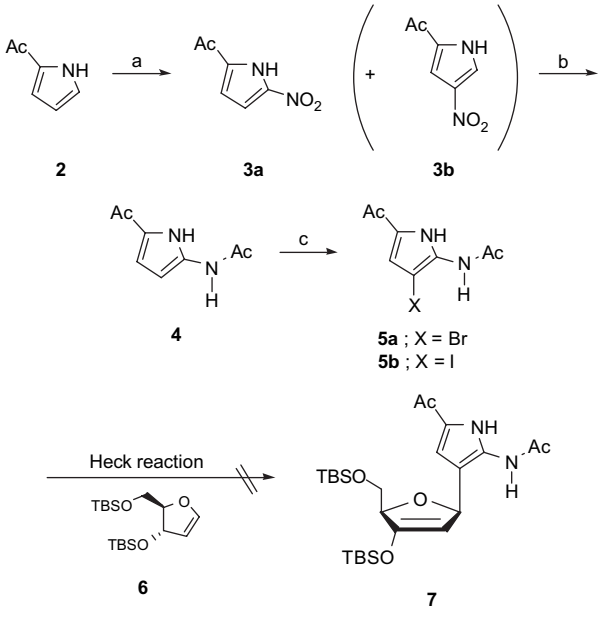


Figure 4. Base pairing of 2-aminopyrrole and $m^{2,2,7}$-G.

influence for the hydrogen bonding pattern in modifying the nucleic acid, there is no problem in the reverse transcription reaction. In the case of mRNAs that contain modified RNA such as trimethylguanosine, the reverse transcription reaction will stop at the position of the modified base. There is a possibility to overcome the problem when triphosphate-form of nucleoside **1** is used in the preparation of the cDNAs.

2. Results and discussion

First, we planned to synthesize **1** by the palladium-catalyzed Heck reaction between nucleobase (or protected) halide and silyl protected furanoid glycal for the construction of the *C*-glycoside bond as a key step of the synthetic strategy as shown in Scheme 1. 5-Acetyl-2-nitro-1*H*-pyrrole (**3a**) was obtained along with 5-acetyl-3-nitro-1*H*-pyrrole (**3b**) by the reaction of 2-acetylpyrrole (**2**) with nitric acid. Compounds **3a** and **3b** could be separated by silica gel column chromatography. Nitro group of **3a** was reduced with iron in acetic acid to afford 5-acetyl-2-acetylaminopyrrole (**4**). This reduction reaction is advantageous for the synthetic strategy at the point of view of the one-pot introduction of the protecting group for the exocyclic amino group of the halo-aminopyrrole. Compound **4** was halogenated by NBS and NIS to obtain bromide (**5a**) and iodide (**5b**), respectively. The furanoid glycal **6** was prepared by a method described in the literature.[7]

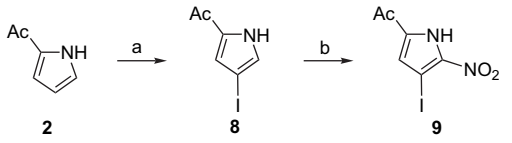


Scheme 1. Reagents and conditions: (a) HNO_3, Ac_2O, −40 °C, 2 h, **3a** (36%), **3b** (51%); (b) Fe, HOAc, 100 °C, 2 h, 36%; (c) NBS, CH_2Cl_2, −40 °C, 1 h, 66% or NIS, CH_2Cl_2, −40 °C, 1 h, 52%.

The crucial step of *C*-deoxyribonucleoside synthesis may often be the *C*-glycosidation reaction by using the Heck reaction. Because troublesome protection/deprotection step is sometimes needed, it is important to avoid further functional group interconversion of the nucleoside. Heck reaction is also convenient to construct the configuration of the β-anomer structure. Therefore, it is strategically superior to construct the Heck reaction of iodide **5** into the synthetic route. However, the Heck reaction between halide **5** and furanoid glycal **6** did not afford the coupling product **7** in spite of the wide investigation of the reaction conditions.[8]

In general, the oxidative addition of the electron rich aryl halide to the palladium(0) species is not so easy to occur. We assumed that the Heck reaction of **5** with **6** did not proceed because electron density over the pyrrole ring of the halide **5** was high, owing to the electron donation of the acetylamino group. Furthermore, there might be a possibility that the catalytic cycle was inhibited because the iodide **5** strongly coordinated to the palladium.

Because the Heck reaction between **5** and **6** did not occur, the synthetic route was changed to carry out the Heck reaction of 5-acetyl-3-iodo-2-nitro-1*H*-pyrrole (**9**) prior to the reduction of the nitro group. The preparation of iodide **9** is shown in Scheme 2. Pyrrole **2** was quantitatively iodinated by NIS to afford 2-acetyl-4-iodo-1*H*-pyrrole (**8**).[9] Compound **9** was obtained by the treatment of **8** with nitric acid in moderate yield.



Scheme 2. Reagents and conditions: (a) NIS, $CHCl_3/CCl_4$, Amberlyst-15DRY, 70 °C, 24 h, 96%; (b) HNO_3, Ac_2O, −40 °C, 2 h, 43%.

The Heck coupling adduct **10** was obtained by the reaction of iodide **9** and furanoid glycal **6** in low yield under conventional conditions (ligand; triphenylphosphine, base; triethylamine, solvent; DMF) using microwave at 140 °C for 10 min. Tri(*o*-tolyl)phosphine was found to be an optimal ligand by the assessment of the reaction condition. Triethylamine and diisopropylethylamine were tolerable as bases, whereas inorganic base such as sodium carbonate or potassium phosphate was not. DMF gave best yield as a solvent. An additive effect is depicted in Table 1. Addition of excess amount of lithium chloride significantly increased the yield of the Heck coupling adduct (entry 18).[10] On the other hand, addition of ammonium salt did not affect the yield even if the quantity was increased.[11]

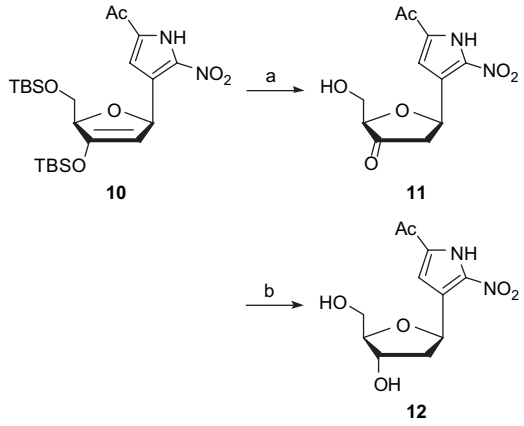
The yield of the Heck reaction between iodide **9** and furanoid glycal **6** was improved by using an excess amount of lithium chloride as an additive. Additives such as inorganic salt generally enhance the reactivity by coordinating the anion to the intermediate palladium species. However, the effect in our case is not clear because the yield was different depending on the counter cations of the additives.

Adduct **10** was desilylated by tetrabutylammonium fluoride (TBAF) to afford 3′-keto nucleoside (**11**) in good yield (Scheme 3). 5-Acetyl-2-nitro-1*H*-pyrrole *C*-deoxyribonucleoside (**12**) was obtained by diastereoselective reduction of 3′-keto group of **11** in high yield.

Table 1. Assessment of additives in the Heck reaction of 5-acetyl-3-iodo-2-nitropyrrole **9** with protected furanoid glycal **6**[a]

Entry	Additive (equiv)	Yield of **10**[b] (%)
1	Me_4NCl (1)	6
2	Me_4NBr (1)	15
3	Me_4NI (1)	9
4	Et_4NCl (1)	19
5	Et_4NBr (1)	8
6	Et_4NI (1)	6
7	Pr_4NCl (1)	9
8	Pr_4NBr (1)	10
9	Pr_4NI (1)	5
10	Bu_4NCl (1)	9
11	Bu_4NBr (1)	18
12	Bu_4NI (1)	4
13	LiCl (1)	19
14	NaCl (1)	17
15	KCl (1)	5
16	KCl+18-C-6 (1)	9
17	LiCl (2)	19
18	LiCl (5)	48
19	LiCl (10)	35
20	None (—)	12

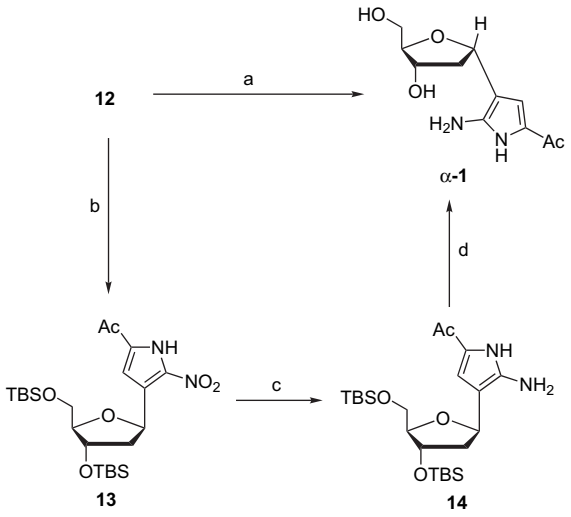
[a] All reactions were carried out using **9** (0.5 mmol), **6** (0.5 mmol), $PdCl_2[P(o\text{-tol})_3]_2$ (10 mol %), Et_3N (4 equiv), and additives in DMF (5 mL) under irradiation of microwave at 140 °C for 10 min.
[b] Isolated yield.



Scheme 3. Reagents and conditions: (a) 70%-$TBAF_{aq}$, HOAc, THF, rt, 10 h, 80%; (b) $NaBH(OAc)_3$, HOAc, MeCN, −40 to 0 °C, 2 h, 87%.

The nitro group of **12** could reduce by the hydrogenation with palladium charcoal in moderate yield. However, the product of the reduction was confirmed as α-anomer that had completely inversed at the anomeric carbon by the measurement of the NOE spectra (Scheme 4). On the other hand, the reduction of compound **13** where the 3′- and 5′-hydroxyl groups were protected by the silyl group occurred almost quantitatively with retention of the stereochemistry, and α-anomer was not detected. Although 3′- and 5′-silyl groups of **14** could not deprotect under usual condition by treatment of TBAF, the removal of the silyl group was achieved by using trimethylsilyl triflate. However, the inversion of the anomeric carbon occurred again, and resulting product was **α-1**.

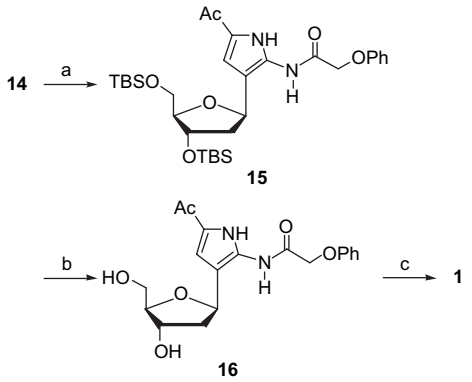
The *C*-nucleoside sometimes causes the inversion of the anomeric carbon by protonation to the anomeric oxygen



Scheme 4. Reagents and conditions: (a) H_2, 10%-Pd/C, H_2O, rt, 40 min, 46%; (b) *tert*-$BuMe_2SiCl$, imidazole, DMF, rt, overnight, 97%; (c) H_2, 10%-Pd/C, MeOH, rt, 2.5 h, 96%; (d) Me_3SiOTf, MeOH, Et_3N, −40 °C, 2 h, 84%.

on the sugar moiety under acidic conditions.[12] The epimerization of *C*-deoxyribonucleoside, in the presence of benzenesulfonic acid and small amounts of water under toluene reflux condition, have been reported by Kool et al.[12a] On the other hand, Benner described that the *C*-nucleoside having hydrogen at the appropriate position of the base moiety epimerize to afford four isomers depending on pH, based on the report concerned with the epimerization of pseudouridine.[12b–d,13] We believe that the epimerization that occurred in deprotecting nucleoside **14** took place by a similar mechanism to the description in Benner's report. To our knowledge, there is no report available that describes inversion of anomeric carbon under the condition of palladium-catalyzed hydrogenation.

To avoid such epimerization, the phenoxyacetyl group that can easily be deprotected under basic conditions was introduced to the amino group of the compound **14** (Scheme 5). The desilylation of compound **15** was smoothly proceeded to afford **16**. Removal of the phenoxyacetyl group with ethylene diamine gave the desired 5-acetyl-2-amino-1*H*-pyrrole *C*-deoxyribonucleoside **1**. The NOE experiments



Scheme 5. Reagents and conditions: (a) $PhOCH_2COCl$, Et_3N, THF, rt,1.5 h, 97%; (b) 70%-$TBAF_{aq}$, HOAc, THF, rt, overnight, 45%; (c) H_2NCH_2-CH_2NH_2, rt, overnight, 50%.

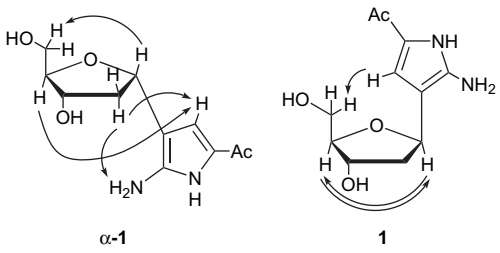


Figure 5. NOE correlations of compound **1** and **α-1**.

revealed that compound **1** has a β-conformation (Fig. 5). No α-anomer was detected.

3. Conclusion

In summary, we achieved the synthesis of the novel *C*-nucleoside, 2-aminopyrrole *C*-deoxyribonucleoside **1**. The nucleoside **1** does not only have a promising ability for molecular biological use, but the synthetic route also includes some synthetically interesting phenomena. The significant additive effect was observed in the *C*-glycosidation step using palladium-catalyzed Heck coupling. To our knowledge, it is unusual that the use of a large excess amount of additive is effective. Although the mechanism is still unclear, epimerization under the condition of hydrogenation catalyzed by palladium is also unusual. A molecular biological study using nucleoside **1** is in progress.

4. Experimental

4.1. General

^1H 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). 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). The reactions using 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 or NH Silica (100–200 mesh) purchased from Fuji Silysia Chemical Ltd. Commercial grade reagents and solvents were used as supplied. Furanoid glycal **6** was prepared according to the method described in the literature.[5] All reactions sensitive to oxygen or moisture were carried out under nitrogen atmosphere.

4.1.1. 5-Acetyl-2-nitro-1*H*-pyrrole (3a).[14] To a solution of **2** (30 mmol, 3.27 g) in acetic anhydride (36 mL) was added dropwise 70%-nitric acid (2.6 mL) at −40 °C over 30 min. The mixture was warmed to room temperature with stirring over 2 h, and then poured into ice-cooled water and extracted with ethyl acetate (3×50 mL). The combined organic layers

were dried over anhydrous magnesium sulfate and evaporated. Flash column chromatography (dichloromethane/ethyl acetate=19:1) of the residue gave **3a** (36%, 1.66 g) and **3b** (51%, 2.36 g) as a pale orange solid. Mp 234 °C: (**3a**) ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.22 (br s, 1H), 7.09 (dd, J= 2.4, 3.9 Hz, 1H), 6.87 (d, J=2.4, 3.9 Hz, 1H); FABMS (*m*/*e*) 155 (MH^+, 3), 93 (100), 76 (38), 58 (25), 46 (20); HRMS (FAB): calcd for $C_6H_7N_2O_3$ $[M+H]^+$ 155.04512, found 155.0464; (**3b**) ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.01 (br s, 1H), 7.83 (dd, J=1.0, 3.4 Hz, 1H), 7.41 (d, J=1.5 Hz, 1H).

4.1.2. 5-Acetyl-2-acetylamino-1*H*-pyrrole (4). A mixture of **3a** (6.4 mmol, 1.00 g), iron (25.8 mmol, 1.44 g), and acetic acid (30 mL) was stirred at 100 °C for 2 h. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography (ethyl acetate) to give **4** as a red-brown solid. Yield 36% (0.385 g): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (br s, 1H), 10.78 (br s, 1H), 6.95 (d, J=3.9 Hz, 1H), 5.84 (d, J=3.9 Hz, 1H), 2.27 (s, 3H), 2.07 (s, 3H); FABMS (*m*/*e*) 167 (MH^+, 73), 154 (100), 107 (58), 89 (50), 78 (47); HRMS (FAB): calcd for $C_8H_{11}N_2O_2$ $[M+H]^+$ 167.08150, found 167.0827.

4.1.3. 5-Acetyl-2-acetylamino-3-bromo-1*H*-pyrrole (5a). To a solution of **4** (0.41 mmol, 0.069 g) in dichloromethane (10 mL) was added *N*-bromosuccinimide (0.5 mmol, 0.089 g) at −40 °C. After stirring for 1 h, the mixture was allowed to warm to room temperature. The mixture was evaporated and purified by flash column chromatography (dichloromethane/ethyl acetate=1:1) to give **5a** as a red-brown solid. Yield 66% (0.066 g), mp 218 °C: ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.91 (br s, 1H), 7.58 (br s, 1H), 6.82 (d, J=2.9 Hz, 1H), 2.36 (s, 3H), 2.26 (s, 3H); FABMS (*m*/*e*) 245 (MH^+ (^{79}Br), 68), 247 (MH^+ (^{81}Br), 67), 154 (100), 59 (78), 70 (66), 45 (58); HRMS (FAB): calcd for $C_8H_{10}BrN_2O_2$ $[M+H]^+$ 244.99202, found 244.9934.

4.1.4. 5-Acetyl-2-acetylamino-3-iodo-1*H*-pyrrole (5b). Compound **5b** was prepared by the similar procedure of **5a** by using *N*-iodosuccinimide instead of *N*-bromosuccinimide (2.3 mmol scale; yield 52%, 0.354 g): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.98 (br s, 1H), 7.49 (br s, 1H), 6.89 (d, J=2.9 Hz, 1H), 2.36 (s, 3H), 2.27 (s, 3H); FABMS (*m*/*e*) 293 (MH^+, 100), 166 (23), 250 (21), 59 (20), 107 (18); HRMS (FAB): calcd for $C_8H_{10}IN_2O_2$ $[M+H]^+$ 292.97815, found 292.9796.

4.1.5. 2-Acetyl-4-iodo-1*H*-pyrrole (8). A mixture of **2** (10 mmol, 1.09 g), Amberlyst-15dry (0.1 g), *N*-iodosuccinimide (10 mmol, 2.25 g) in chloroform/carbon tetrachloride (1/1, 40 mL) was stirred at 70 °C for 24 h. The Amberlyst-15dry was filtered off and the solvent was removed under reduced pressure. Flash column chromatography (*n*-hexane/ethyl acetate=7:3) of the residue gave **8** as a pale gray solid. Yield 96% (2.27 g), mp 235 °C: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.71 (br s, 1H), 7.08 (dd, J=1.5, 2.9 Hz, 1H), 6.99 (dd, J=1.5, 2.9 Hz, 1H), 2.42 (s, 3H); FABMS (*m*/*e*) 236 (MH^+, 13), 154 (100), 107 (20), 90 (19), 78 (18); HRMS (FAB): calcd for C_6H_6INO $[M+H]^+$ 235.95669, found 235.9590.

4.1.6. 5-Acetyl-3-iodo-2-nitro-1*H*-pyrrole (9). Compound **9** was prepared from **8** (9.3 mmol, 2.20 g) by the similar procedure of the preparation of **3a**. Purification was carried out by flash column chromatography (*n*-hexane/ethyl acetate=5:3). The product was obtained as an orange solid. Yield 43% (1.11 g): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.27 (br s, 1H), 7.08 (d, J=2.9 Hz, 1H), 2.52 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 188.7, 139.9, 134.4, 124.8, 67.5, 26.7; FABMS (*m*/*e*) 281 (MH^+, 54), 59 (100), 43 (78), 70 (74), 265 (25); HRMS (FAB): calcd for $C_6H_6IN_2O_3$ $[M+H]^+$ 280.94177, found 280.9420.

4.1.7. Palladium-catalyzed Heck coupling between 9 and furanoid glycal 6. A reaction tube containing a mixture of **9** (0.5 mmol, 0.140 g), dichlorobis[tri(*o*-tolyl)phosphine]palladium (10 mol %, 79 mg), furanoid glycal **6** (0.5 mmol, 0.172 g), triethylamine (2 mmol, 0.202 g), and additive in DMF (5 mL/mmol) was sealed. The mixture was heated using a microwave at 140 °C for 10 min. The solvent was removed under reduced pressure and then the residue was purified by flash column chromatography (*n*-hexane/ethyl acetate=4:1). The adduct **10** was obtained as a pale yellow oil (0.119 g, 48% in the presence of 2.5 mmol of lithium chloride as an additive): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.96 (br s, 1H), 7.14 (s, 1H), 6.22 (d, J=3.4 Hz, 1H), 4.93 (s, 1H), 4.63–4.58 (m, 1H), 3.95 (d, J=11.2 Hz, 1H), 3.78 (dd, J=3.9, 11.2 Hz, 1H), 2.49 (s, 3H), 0.94 (s, 9H), 0.89 (s, 9H), 0.23 (s, 3H), 0.18 (s, 3H), 0.09 (s, 3H), 0.07 (s, 3H); FABMS (*m*/*e*) 497 (MH^+, 11), 74 (100), 147 (43), 233 (14), 479 (11); HRMS (FAB): calcd for $C_{23}H_{41}N_2O_6Si_2$ $[M+H]^+$ 497.24977, found 497.2491.

4.1.8. 2*R*,5*R*-5-(5-Acetyl-2-nitro-1*H*-pyrrol-3-yl)-2-hydroxymethyltetrahydrofuran-3-one (11). A mixture of **10** (1.6 mmol, 0.793 g), 75%-tetrabutylammonium fluoride (6.4 mmol, 2.02 g), acetic acid (12.8 mmol, 0.763 g), and THF (16 mL) was stirred at room temperature overnight. After concentration in vacuo, the product was purified by flash column chromatography (ethyl acetate) to give **11** as a pale yellow solid. Yield 80% (0.344 g), mp 240 °C: ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.69 (s, 1H), 7.39 (s, 1H), 5.66 (dd, J=6.3, 10.7 Hz, 1H), 5.02 (br s, 1H), 4.04 (t, J=2.9 Hz, 1H), 3.73 (dd, J=2.4, 12.2 Hz, 1H), 3.64 (dd, J=3.4, 12.2 Hz, 1H), 2.92 (dd, J=6.3, 17.6 Hz, 1H), 2.52 (s, 3H), 2.31 (dd, J=10.7, 17.6 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 213.8, 189.1, 135.9, 132.5, 127.9, 114.3, 82.3, 70.1, 60.3, 43.6, 26.7; HRMS (FAB): calcd for $C_{11}H_{13}N_2O_6$ $[M+H]^+$ 269.07681, found 269.0790.

4.1.9. 5-Acetyl-3-(2′-deoxy-β-D-ribofuranosyl)-2-nitro-1*H*-pyrrole (12). To a stirring solution of **11** (4.8 mmol, 1.29 g) in acetic acid (29 mL) and acetonitrile (96 mL), sodium tri(acetoxy)borohydride (5.3 mmol, 1.124 g) 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 residue was purified by flash column chromatography (ethyl acetate/methanol=9:1) to give **12** as a pale yellow solid. Yield 87% (1.13 g), mp 165 °C (decomposed): ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.50 (s, 1H), 7.13 (s, 1H), 5.44 (dd, J=5.9, 9.8 Hz, 1H), 5.08 (br s, 1H), 4.79 (br s, 1H), 4.17 (td, J=2.4, 5.4 Hz, 1H), 3.77 (dt, J=2.4, 4.9 Hz, 1H), 3.53–3.43 (m, 2H), 2.49 (s, 3H), 2.25 (ddd, J=2.0, 5.4, 12.2 Hz, 1H), 1.74 (ddd, J=5.9, 9.8, 12.7 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.1, 135.5, 132.3, 130.1,

114.0, 87.5, 72.9, 72.0, 62.2, 41.5, 26.7; HRMS (FAB): calcd for $C_{11}H_{15}N_2O_6$ $[M+H]^+$ 271.09246, found 271.0938.

4.1.10. 5-Acetyl-3-[3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxy-β-D-ribofuranosyl]-2-nitro-1*H*-pyrrole (13). A solution of **12** (3.9 mmol, 1.04 g), imidazole (24 mmol, 1.63 g), and *tert*-butyldimethylchlorosilane (9.7 mmol, 1.47 g) in DMF (20 mL) was stirred at room temperature overnight. To the mixture was added ethyl acetate (100 mL) and washed with water (3×100 mL) and brine (100 mL). The organic layer was dried over magnesium sulfate and evaporated. Flash column chromatography (*n*-hexane/ethyl acetate=10:1) of the residue gave **13** as a yellow oil. Yield 97% (1.86 g): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.02 (br s, 1H), 7.03 (d, J=3.4 Hz, 1H), 5.64 (dd, J=5.4, 9.8 Hz, 1H), 4.40 (dt, J=2.4, 5.4 Hz, 1H), 3.99–3.95 (m, 1H), 3.78 (dd, J=3.9, 11.2 Hz, 1H), 3.70 (dd, J=4.9, 11.2 Hz, 1H), 2.50 (s, 3H), 2.42 (ddd, J=2.0, 5.9, 12.7 Hz, 1H), 1.77 (ddd, J=5.4, 9.8, 12.7 Hz, 1H), 0.92 (s, 9H), 0.91 (s, 9H), 0.112 (s, 3H), 0.106 (s, 3H), 0.104 (s, 3H), 0.095 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.6, 130.5, 113.6, 88.0, 77.2, 74.0, 73.5, 63.5, 42.2, 25.91, 25.88, 25.8, 25.5, −4.6, −4.8, −5.37, −5.42; HRMS (FAB): calcd for $C_{23}H_{43}N_2O_6Si_2$ $[M+H]^+$ 499.26542, found 499.2647.

4.1.11. 5-Acetyl-2-amino-3-[3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxy-β-D-ribofuranosyl]-1*H*-pyrrole (14). A solution of **13** (3.2 mmol, 1.61 g) in methanol (80 mL) was reduced over 10% palladium on carbon (0.17 mmol, 0.18 g) under hydrogen (1 atm, balloon) at room temperature for 2 h. Removal of the catalyst and evaporation of the solvent under reduced pressure gave **14** as a yellow oil. Yield 96% (1.46 g): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.45 (br s, 1H), 6.72 (s, 1H), 5.05 (dd, J=5.6, 10.8 Hz, 1H), 4.99 (br s, 2H), 4.38 (d, J=6.0 Hz, 1H), 3.87 (d, J=2.0 Hz, 1H), 3.78 (dd, J=3.2, 11.2 Hz, 1H), 3.74 (dd, J=3.2, 11.2 Hz, 1H), 2.24 (s, 3H), 2.11 (ddd, J=6.0, 10.8, 12.8 Hz, 1H), 1.93 (dd, J=5.6, 12.8 Hz, 1H), 0.912 (s, 9H), 0.907 (s, 9H), 0.093 (s, 3H), 0.084 (s, 3H), 0.079 (s, 6H); HRMS (FAB): calcd for $C_{23}H_{45}N_2O_4Si_2$ $[M+H]^+$ 469.29124, found 469.2933.

4.1.12. 5-Acetyl-2-amino-3-(2′-deoxy-α-D-ribofuranosyl)-1*H*-pyrrole (α-1). (Scheme 4, path a): A solution of **12** (2.7 mmol, 0.74 g) in water (30 mL) was reduced over 10% palladium on carbon (0.27 mmol, 0.29 g) under hydrogen (1 atm, balloon) at room temperature for 40 min. Removal of the catalyst and evaporation of the solvent under reduced pressure gave an oil. The resulting oil was purified by flash column chromatography (NH silica, ethyl acetate/methanol=3:2) to give **α-1** as a yellow oil. Yield 46% (0.30 g): ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.30 (s, 1H), 6.71 (d, J=2.4 Hz, 1H), 5.10 (s, 2H), 4.54 (d, J=6.4 Hz, 1H), 4.35 (d, J=4.4 Hz, 1H), 4.16 (dd, J=2.0, 11.2 Hz, 1H), 3.76 (dd, J=2.0, 12.2 Hz, 1H), 3.65 (d, J=12.2 Hz, 1H), 3.64–3.56 (m, 1H), 3.55 (br s, 1H), 2.11 (s, 3H), 1.90 (dd, J=11.7, 23.9 Hz, 1H), 1.62–1.54 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.2, 144.2, 122.5, 118.3, 107.0, 70.7, 70.2, 68.5, 67.2, 35.0, 20.8; HRMS (FAB): calcd for $C_{11}H_{17}N_2O_4$ $[M+H]^+$ 241.11828, found 241.1197.

(Scheme 4, path d): To a solution of **12** (0.14 mmol, 0.065 g) in dichloromethane (2 mL) was cooled at −40 °C and added trimethylsilyl triflate (2.8 mmol, 0.5 mL). After stirring for 12 h at −40 °C, methanol (2 mL) was added and the mixture was stirred for 4 h. To quench the reaction, triethylamine (0.5 mL) was added and the mixture was stirred for 2 h. The solvent was evaporated, and the residue was purified by silica gel column chromatography (NH silica, ethyl acetate/methanol=3:2) to give **α-1**. Yield 84% (0.028 g).

4.1.13. 5-Acetyl-3-[3′,5′-bis(*tert*-butyldimethylsilyl)-2′-deoxy-β-D-ribofuranosyl]-2-phenoxyacetylamino-1*H*-pyrrole (15). To a solution of **14** (2.8 mmol, 1.33 g) and triethylamine (4.2 mmol, 0.59 mL) in dry-THF (40 mL) was added dropwise phenoxyacetyl chloride (3.4 mmol, 0.47 mL). After being stirred at room temperature for 90 min, the mixture was poured into water (30 mL) and extracted with ethyl acetate (3×30 mL). The combined organic layers were dried over magnesium sulfate and concentrated. Flash column chromatography (dichloromethane/methanol=29:1) of the residue gave **15** as a yellow oil. Yield 97% (1.66 g): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.89 (br s, 1H), 10.15 (br s, 1H), 7.37–7.31 (m, 2H), 7.06 (t, J=7.2 Hz, 1H), 6.98–6.93 (m, 2H), 6.56 (d, J=3.2 Hz, 1H), 5.23 (dd, J=5.6, 11.2 Hz, 1H), 4.61 (d, J=6.4 Hz, 1H), 4.38 (d, J=4.8 Hz, 1H), 4.02–3.98 (m, 1H), 3.66 (dd, J=4.0, 10.8 Hz, 1H), 3.44 (dd, J=6.8, 10.8 Hz, 1H), 2.35 (s, 3H), 2.22–2.16 (m, 1H), 1.92 (ddd, J=5.2, 10.8, 12.4 Hz, 1H), 0.92 (s, 9H), 0.80 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H), −0.06 (s, 3H), −0.10 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.9, 166.1, 131.3, 129.9, 126.0, 122.4, 114.8, 113.5, 88.5, 86.9, 75.5, 74.1, 67.1, 63.6, 63.1, 42.7, 25.7, 25.4, 24.8, 18.2, 18.0, 13.1, −4.7, −5.5; HRMS (FAB): calcd for $C_{31}H_{51}N_2O_6Si_2$ $[M+H]^+$ 603.32802, found 603.3309.

4.1.14. 5-Acetyl-3-[2′-deoxy-β-D-ribofuranosyl]-2-phenoxyacetylamino-1*H*-pyrrole (16). Compound **16** was prepared from **15** (2.0 mmol, 1.18 g) by the similar procedure of the preparation of **11**. Purification was carried out by flash column chromatography (ethyl acetate). The product was obtained as a pale yellow solid. Yield 45% (0.33 g), mp 182 °C: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.18 (br s, 1H), 10.44 (br s, 1H), 7.34–7.29 (m, 2H), 7.04–6.96 (m, 4H), 5.08–5.00 (m, 2H), 4.75 (s, 2H), 4.21 (br s, 1H), 3.76 (br s, 1H), 3.55–3.45 (m, 2H), 2.28 (s, 3H), 2.01–1.88 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.7, 167.3, 130.6, 130.1, 129.6, 126.0, 121.4, 114.7, 114.6, 71.0, 68.1, 67.1, 66.3, 35.7, 24.8; HRMS (FAB): calcd for $C_{19}H_{23}N_2O_6$ $[M+H]^+$ 375.15506, found 375.1570.

4.1.15. 5-Acetyl-2-amino-3-(2′-deoxy-β-D-ribofuranosyl)-1*H*-pyrrole (1). A solution of **16** (0.5 mmol, 0.19 g) in ethylene diamine (5 mL) was stirred at room temperature overnight and then concentrated under reduced pressure. The resulting residue was purified by flash column chromatography (NH silica, ethyl acetate/methanol=7:1 to 3:1 gradient) to give **1** as a yellow oil. Yield 50% (0.06 g): ^{1}H NMR (400 MHz, CD_3OD) δ 7.02 (s, 1H), 5.06 (dd, J=5.4, 11.2 Hz, 1H), 4.37 (d, J=5.9 Hz, 1H), 3.90 (d, J=2.4 Hz, 1H), 3.68 (d, J=3.9 Hz, 2H), 2.23 (s, 3H), 2.21–2.16 (m, 1H) 1.93 (dd, J=5.4, 13.2 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 183.7, 147.8, 123.9, 123.2, 108.4, 88.8, 76.2, 74.7, 63.7, 42.2, 23.6; HRMS (FAB): calcd for $C_{11}H_{17}N_2O_4$ $[M+H]^+$ 241.11828, found 241.1168.

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