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Efficient synthesis of 2′-*C*-α-aminomethyl-2′-deoxynucleosides†‡§

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Starting from methyl 3,5-di-*O*-benzyl-2-keto-α-D-ribofuranoside, a convergent, six-step synthesis is developed to give efficiently all four 2′-*C*-α-aminomethyl-2′-deoxynucleosides (U, C, A, G) in 38%, 42%, 12%, 12% yield, respectively. Convergence is achieved by the glycosylation of persilylated nucleobases with methyl 2-α-phthalimidomethyl ribofuranoside.

The chemical synthesis of modified nucleosides and the incorporation of nonnatural nucleosides into RNA have become increasingly important in medical applications and as reagents for structure and function investigations of RNAs.[1,2] A new class of nucleoside analogues, 2′-*C*-α-aminomethyl-2′-deoxynucleosides (Fig. 1, **IV**), which hold both amino and 2′-alkyl functionalities, have features common to both 2′-α-amino-2′-deoxynucleosides[3] and 2′-*C*-branched nucleosides,[4–8] and therefore might possess interesting antiviral and anticancer activities. Additionally, oligonucleotides containing 2′-aminomethyl nucleosides provide a new opportunity for regiospecific chemoligation reaction with a variety of amine-reactive probes to attach desired reporter groups.[9,10] Here we describe an efficient approach to all of the four potentially important nucleoside analogues: 2′-*C*-α-aminomethyl-2′-deoxynucleosides (A, C, G, U).

We designed a convergent approach (glycosylation of various nucleobases with 2′-aminomethyl ribose derivatives) to prepare the four 2′-*C*-α-aminomethylnucleosides (U, C, A and G) efficiently. We converted methyl 3,5-di-*O*-benzyl-2-keto-α-D-ribofuranoside (**1**)[11] in two steps (*via* Wittig and hydroboration reactions) to 2-α-hydroxymethyl ribofuranose derivative **2** in 52% yield (Scheme 1). Under Mitsunobu conditions,[12] **2** reacts to form 2-α-phthalimidomethyl ribofuranoside **3** in 90% yield. Deprotection of **3** with methylamine in ethanol[13] followed by reaction with *S*-ethyl α,α,α-trifluoroacetate gives 2-α-(α,α,α-trifluoroacetyl)-aminomethyl ribofuranoside **4** in 68% yield.

Glycosylation of persilylated pyrimidine nucleobases (uracil and N^4-acetylcytosine) with 2′-aminomethyl ribofuranoside derivatives **3** and **4** in the presence of $SnCl_4$ gives the corresponding uridine and cytidine derivatives with high β-selectivity (91 : 9 to 98 : 2) (Scheme 2). Although yield and β-selectivity were higher using 2-α-phthalimidomethyl ribofuranoside **3**, the high β-selectivity of the glycosylation reaction indicates that both phthalyl and trifluoroacetyl groups can stabilize the 1-oxocarbenium ion intermediates and direct the nucleobase to attack the β face of the ribofuranoside.

Glycosylation of purine nucleobases generally requires nonpolar solvent and the presence of TMSOTf to achieve the optimized yield and β-9 selectivity.[14,15] Glycosylation of persilylated

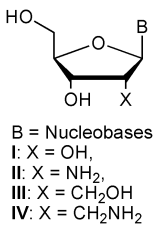
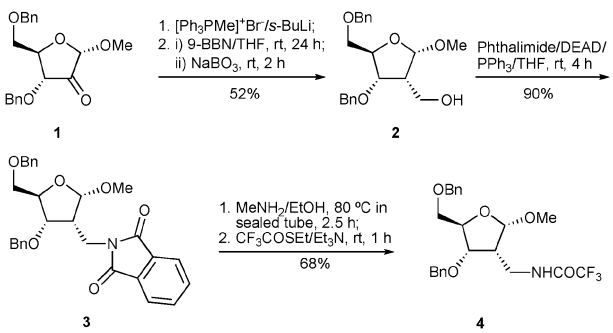
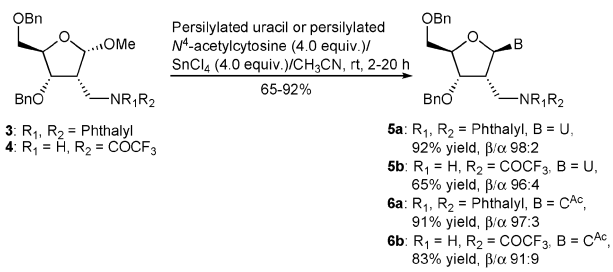


Fig. 1 Structures of ribonucleosides (**I**) and modified nucleosides (**II–IV**).



Scheme 1 Synthesis of glycosylation reagents **3** and **4**.



Scheme 2 Glycosylation of persilylated pyrimidine nucleobases with **3** and **4**.

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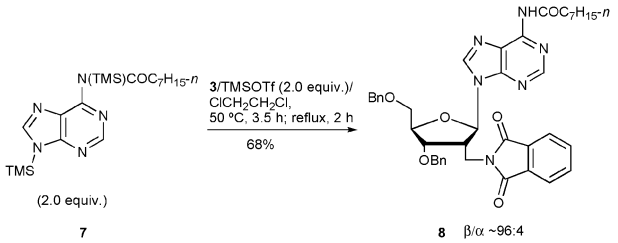
Table 1 Synthesis of **8** by the glycosylation of **7** with **3**



Entry	Solvent	Reaction conditions	Yield (%)	β/α
1	$ClCH_2CH_2Cl$	Reflux, 2 h	71	87 : 13
2	$ClCH_2CH_2Cl$	rt, 48 h; reflux, 1 h	68	91 : 9
3[a]	CH_3CN	Reflux, 1.5 h	—	—
4	$ClCH_2CH_2Cl$	50 °C, 3.5 h; reflux, 2 h	68	96 : 4

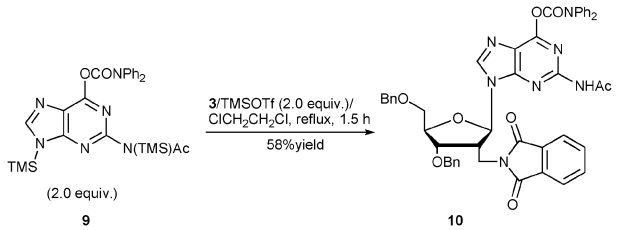
[a] Lower yield and lower selectivity.

N^6-benzoyladenosine with **3** or **4** in the presence of TMSOTf in refluxing 1,2-dichloroethane for two hours yields the corresponding products in low yields (only ~35%) and moderate selectivity, with **3** giving better β-selectivity than with **4** (β/α 80 : 20 and 65 : 35, respectively). N^6-Octanoyladenosine has been used in the glycosylation with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose for its better solubility than N^6-benzoyladenosine.[16] It also has been used in the transglycosylation of 2′-α-trifluoroacetylaminouridine.[17] We then found that use of persilylated N^6-octanoyladenosine **7** significantly improved yield and β-selectivity of the glycosylation with **3**. The results are shown in Table 1. Glycosylation in nonpolar solvent (1,2-dichloroethane) generally yields the glycosylation product in high β-selectivity. In contrast, glycosylation in the polar solvent, acetonitrile, gave the product in low yield and low selectivity (Table 1, Entry 3). The optimized results with 68% yield and ~96 : 4 β-selectivity were achieved using 1,2-dichloroethane at 50 °C for 3.5 hours, then at reflux for 2 hours (Table 1, entry 4).

The glycosylation of persilylated N^2-acetylguanosine (2 equiv.) with glycosylating agent **3** in the presence of TMSOTf (2 equiv.) gave complex results even in nonpolar solvents such as 1,2-dichloroethane, toluene and benzene. Reactions gave all of four possible products (β-9, β-7, α-9 and α-7) in low yields and in low selectivity. However, using persilylated N^2-acetyl-O^6-(diphenylcarbamoyl)-guanine (**9**)[18,19] glycosylation in the presence of TMSOTf gave the β-9 isomer product **10** exclusively. The glycosylation of persilylated N^2-acetyl-O^6-(diphenylcarbamoyl)-guanine (**9**) with **3** under various conditions are shown in Table 2. The optimized yield (58%) is obtained if the glycosylation of N^2-acetyl-O^6-(diphenylcarbamoyl)-guanine (**9**) with **3** is carried out in refluxing 1,2-dichloroethane (80 °C oil bath) for 1.5 hours (Table 2, entry 5).

To remove the 3′,5′-di-*O*-benzyl groups from the glycosylation products, we tested two debenzylation methods (Method A: $Pd(OH)_2$ or Pd catalyzed hydrogenation,[20] and Method B: boron trichloride (Lewis acid) induced benzyl ether cleavage[21–23]) (Table 3). Method A removed the benzyl groups from uridine derivatives **5a** and **5b** to give compounds **11a** and **11b** in 62% and 81% yield, respectively (Table 3, entries 1, 3). The uridine derivative **5a** can also be debenzylated by Method B to give compound **11a** in 90% yield (entry 2). However, for the cytidine

Table 2 Synthesis of **10** by the glycosylation of **9** with **3**



Entry	Solvent	Reaction conditions	Yield (%)
1	$ClCH_2CH_2Cl$	rt, 62 h; reflux, 1 h	45
2	Toluene	80 °C, 3 h	15
3[a]	$ClCH_2CH_2Cl$	reflux, 4 h	—
4[b]	CH_2Cl_2	reflux, 4 h	—
5	$ClCH_2CH_2Cl$	reflux (80 °C), 1.5 h	58

[a] Product is decomposed significantly and the yield is not determined. [b] Reaction is incomplete and the yield is not determined.

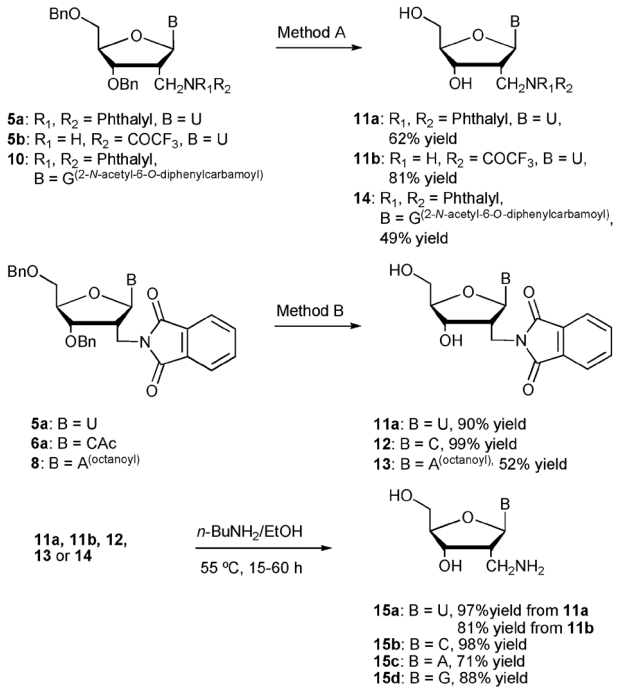
Table 3 Removal of the 3′,5′-di-*O*-benzyl groups of various glycosylation products

Entry	S[a]	Method	Reaction conditions	P[b]	Yield (%)
1	**5a**	A	20% $Pd(OH)_2/C$, H_2, $CH_3CO_2C_2H_5$, rt, 22 h	**11a**	62
2	**5a**	B	1. BCl_3/CH_2Cl_2, −78 °C, 4 h; 2. MeOH/CH_2Cl_2 (v/v 1 : 1), −78 °C-rt, 30 min	**11a**	90
3	**5b**	A	20% $Pd(OH)_2/C$, H_2, $CH_3CO_2C_2H_5$, rt, 17 h	**11b**	81
4	**6a**	B	1. BCl_3/CH_2Cl_2, −78 °C, 2.5 h; 0 °C, 30 min; 2. MeOH, 0 °C-rt, 12 h	**12**	99
5[c]	**6b**	A	20% $Pd(OH)_2/C$, H_2, $CH_3CO_2C_2H_5$, rt, 6 h	—	—
6[d]	**8**	A	20% $Pd(OH)_2/C$, H_2, $CH_3CO_2C_2H_5$, rt, 15 h	—	—
7[e]	**8**	B	1. BCl_3/CH_2Cl_2, −78 °C, 1 h; 2. MeOH/CH_2Cl_2 (v/v 1 : 1), −78 °C-rt, 30 min	—	—
8	**8**	B	1. BCl_3/CH_2Cl_2, −78 °C, 1 h; 2. Et_3N/MeOH/CH_2Cl_2, 78 °C-rt, 30 min	**13**	47
9	**8**	B	1. BCl_3/CH_2Cl_2, −78 °C, 1 h; 2. $NaHCO_3$ (aq.), 78 °C-rt, 30 min	**13**	52
10	**10**	A	20% $Pd(OH)_2/C$, H_2, $CH_3CO_2C_2H_5$, rt, 15 h	**14**	49
11	**10**	A	5% Pd/C, H_2, $CH_3CO_2C_2H_5$, rt, 15 h	**14**	49
12	**10**	B	1. BCl_3/CH_2Cl_2, −78 °C, 1 h; 2. Et_3N/MeOH/CH_2Cl_2, 78 °C-rt, 30 min	**14**	19

[a] S: Starting materials (substrates). [b] P: Products. [c] Cytosine ring also reduced. [d] The results are complicated. [e] Product is decomposed.

derivatives (**6a** and **6b**), Method A in addition to the debenzylation, reduced the 5,6-double bond of the cytosine ring (entry 5). In contrast, Method B removes the benzyl and acetyl groups from cytidine derivative **6a** without affecting the 5,6-double bond to generate **12** in 99% yield (entry 4).

Attempts to remove the benzyl groups from adenosine derivative **8** by Method A gave complex results (entry 6). However, for Method B, if a base such as triethylamine or aqueous sodium bicarbonate was added to quench the reaction (entries 8, 9), the adenosine derivative **8** undergoes selective debenzylation to give compound **13** in 47–52% yield, Without the base, the product



Scheme 3 Synthesis of 2′-α-aminomethyl-2′-deoxynucleosides.

decomposes (entry 7). For the guanosine derivative **10** under the same conditions, Method B gives the corresponding debenzylated product **14** in only 19% yield (entry 12). In contrast, Method A converts the guanosine derivative **10** to compound **14** in 49% yield (entries 10, 11). To complete the synthesis, **11a**, **11b**, **12**, **13** and **14** were treated with *n*-butylamine in ethanol at 55 °C to give the final 2′-*C*-α-aminomethyl-2′-deoxynucleosides **15** (A, C, G, U) in 71–97% yields (Scheme 3).

NOESY experiments confirmed the structures of β-anomers. For the uridine derivative **5a**, we observed strong NOE between 2′-H (δ 3.02) and 3′-H (δ 4.22), between 1′-H (δ 6.14) and CH_2N (δ 4.00) but no NOE between 1′-H and 2′-H. For cytidine derivative **6a**, we observed strong NOE between between 1′-H (δ 6.04) and CH_2N (δ 4.00) but no NOE between 1′-H and 2′-H (δ 2.97). For adenosine derivative **8**, we also observed strong NOE between 1′-H (δ 6.34) and CH_2N (δ 4.16) but only weak NOE between 1′-H and 2′-H (δ 3.84). For 2′-*C*-α-aminomethyl-2′-deoxyguanosine (**15d**), we observed strong NOE between 1′-H (δ 5.81) and 4′-H (δ 4.08), but no NOE between 1′-H (δ 5.81) and 3′-H (δ 4.48); we also observed stronger NOE between 1′-H (δ 5.81) and CH_2N (δ 3.30) but weak NOE between 1′-H (δ 5.81) and 2′-H (δ 3.12). These data suggest that 1′-H and 2′-CH_2N reside on the same face of the ribose ring and therefore the glycosylation products have the β-configuration.

In summary we have developed a novel synthetic approach to a new class of nucleoside analogues: all four 2′-*C*-α-aminomethyl-2′-deoxynucleosides (A, C, G, U) efficiently and convergently synthesized by the glycosylation of persilylated nucleobases with 2-α-phthalimidomethyl ribofuranoside **3** or 2-α-trifluoroacetylaminomethyl ribofuranoside **4**. As glycosylating reagent, 2-α-phthalimidomethyl ribofuranoside **3** gives better results (yield and selectivity) than 2-α-trifluoroacetylaminomethyl ribofuranoside **4**. Debenzylation of the glycosylation products was accomplished either by hydrogenation (Method A) for guanosine derivatives or by boron trichloride induced benzyl ether cleavage (Method B) for uridine, cytidine and adenosine derivatives. These analogues will enable new strategies for investigating structure-function relationships in RNA.

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