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One-step syntheses of alkyl glycosides and alkyl-substituted DNA oligomers by chemoselective glycosidations using DNA bases

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Dedicated to Professor Kuniaki Tatsuta on the occasion of his 65th birthday

Abstract—The simple and practical synthesis of alkyl glycosides by novel chemoselective glycosidations using natural resources, DNA and RNA nucleosides, was realized, and the one-step synthesis of chemoselectively modified DNA oligomers using the glycosidation method was also demonstrated.

Glycosides, also known as glycosubstances, are found in many biologically important molecules such as glycoproteins, glycolipids and antibiotics. Furthermore, some glycosides have appeared as new functional materials. Typically, certain alkyl glycosides are now expected to be biodegradable surfactants and are in demand in many industrial fields. For these reasons, carbohydrates containing alkyl glycosides continue to be the central focus of research in chemistry, biology and material science.[1] One of the most important and fundamental reactions for preparing such glycosides is chemical glycosidation, which is very useful for synthesizing both natural and unnatural ones.[2] Although many glycosidation methods have been developed to improve the chemical yield and the stereoselectivity, the investigation of efficient and practical glycosidation methods is becoming more and more important in synthetic organic chemistry, and urgently needed both in the laboratory and in industry. One of the most important and challenging tasks confronting chemical glycosidations is the simple preparation of a suitable glycosyl donor and its effective use for chemical glycosidation. In general, the preparation of an appropriately functionalized glycosyl donor for chemical glycosidation is not very easy and usually requires multiple operations. Therefore, if such a glycosyl donor could be easily and directly available from nature, that is, natural resources, it would be a great advantage for an efficient and practical chemical glycosidation. In this study, we used DNA as the glycosyl donor. Large amounts of DNA are readily available from natural resources such as salmon albino, whose effective use has not yet been completely determined. In this letter, we disclose, for the first time, the simple and practical synthesis of alkyl glycosides and alkyl-substituted DNA oligomers by chemoselective glycosidations using DNA bases and DNA oligomers (Fig. 1).

To realize our purpose, we noted the G (guanine)-reaction in the Maxam–Gilbert protocol, which is a well known and widely used DNA sequencing method in molecular biology.[3] In the G-reactions, the G base is selectively modified by dimethylsulfate (Me_2SO_4), followed by treatment using hot piperidine, to afford DNA fragments, which are selectively cleaved at the G sites. Based on this result, we expected that the following chemical reaction must take place during the G-reaction of the Maxam–Gilbert protocol as shown in Figure 2. Thus, the G base is selectively alkylated by dimethylsulfate at the *N*-7 position, whose HOMO level is very high,[4] and then the positively charged methylated G base is formed. Next, release of the resulting positively charged G base as the neutral 7-*N*-methylguanine provides the oxonium cation intermediate. Furthermore, water attacks the oxonium intermediate to give the hemiacetal, which reacts with piperidine to form the imine product. The generating imine is labile under basic

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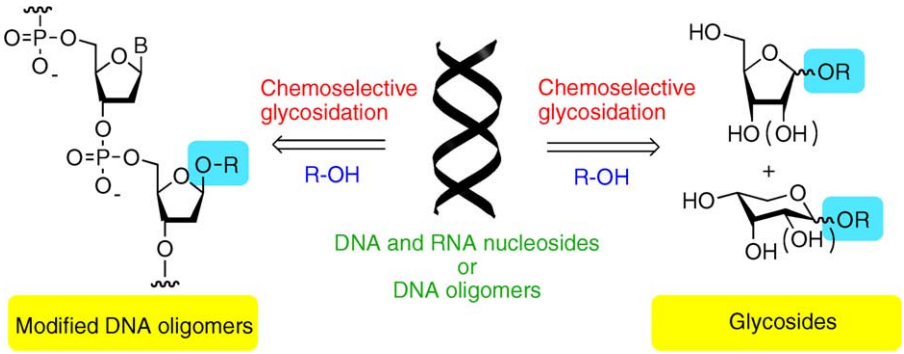


Figure 1. Syntheses of glycosides and modified DNA oligomers by glycosidations using DNA bases and DNA oligomers.

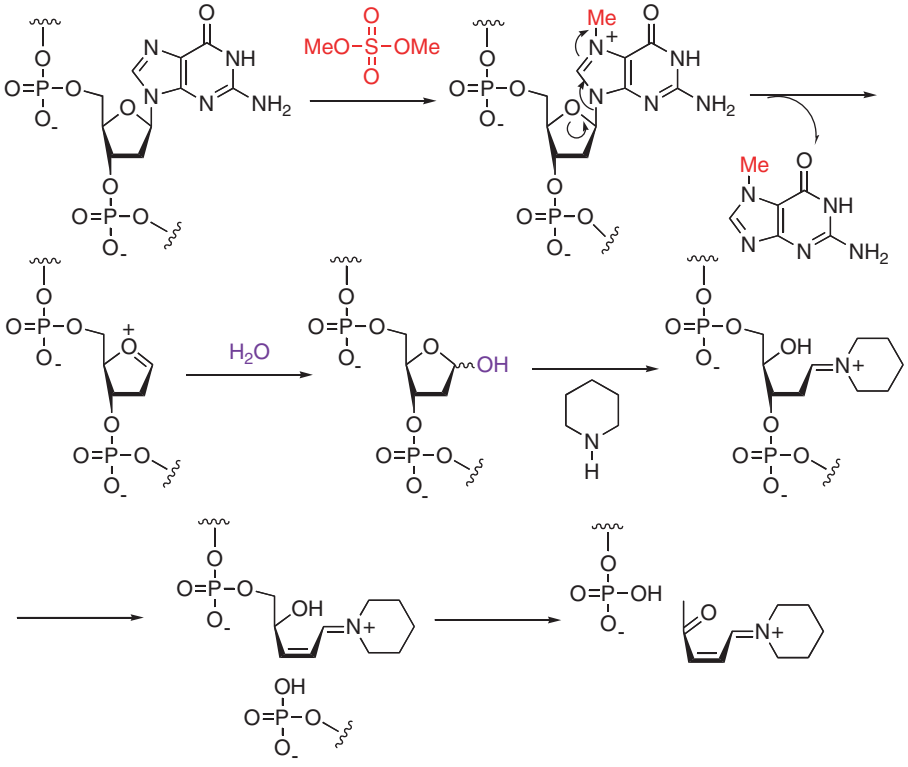


Figure 2. Maxam–Gilbert DNA sequencing method and the presumed mechanism of the G-reaction.

conditions and leads to the DNA cleavage by β-elimination of the phosphate moiety.[3] If this reaction mechanism is correct, when we use an alcohol instead of water under anhydrous conditions, the corresponding glycoside would be obtained in a similar way.

Based on our hypothesis, we first examined the glycosidations using guanosine (**1**), in which the G base is connected with ribose at the C1 position. At this stage, we checked the solubility of the glycosyl donor **1** without any protecting groups in several solvents, and confirmed that **1** is soluble in only DMSO and DMF, and insoluble in PhMe, Et_2O, CH_2Cl_2, MeCN and THF, which were widely used in many conventional chemical glycosidation reactions, due to the high polarity of **1**. For the activating reagents, we tested several alkylating agents such as MeI, MeOTf and BnBr. We found that the glycosidation of **1** and *n*-BuOH (**2**) using MeOTf in DMSO at 80 °C for 5 h smoothly proceeded to give the glycosides **3** together with the corresponding pyranosides **4** in high yield (Fig. 3). It was confirmed that the production of the pyranosides **4** came from the equilibrium between **3** and **4** under the reaction conditions. In addition, we confirmed that MeOTf was superior to the other alkylating agents such as MeI and BnBr, and DMSO was better than DMF as the solvent. In this case, since the unprotected **1** was used as the glycosyl donor, an excess amount of the glycosyl acceptor **2** was needed for obtaining the glycosides **3** and **4** in high yield, because **1** itself has a highly reactive primary hydroxyl group at the C5′-position.

Considering the G+A (adenine)-reaction using formic acid (HCOOH) as the modifying agent in the Maxam–Gilbert protocol,[3] we expected that the glycosidation using **1** would be realized using protic acids. Therefore,

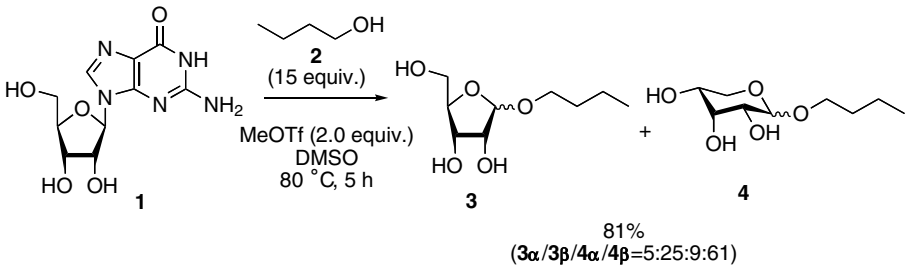


Figure 3. Synthesis of glycosides by the alkylative glycosidation of guanosine (**1**).

we next examined the glycosidations of **1** and **2** using several protic acids such as HCOOH, CSA and TfOH. We found that the glycosidation of **1** and **2** using TfOH in DMSO at 60 °C for 10 h was effectively performed to give the corresponding glycosides **3** and **4** in high yield (Fig. 4). It was confirmed that TfOH was superior to the other protic acids such as HCOOH and CSA. It was noteworthy that although many activating agents of conventional chemical glycosidations such as Lewis and protic acids were significantly deactivated in DMSO, the alkylating agent, MeOTf, and the protic acid, TfOH, worked very well even in DMSO probably due to their high affinity with the nitrogen atom at the 7-position of the G base, whose HOMO level was very high.

We next examined the chemoselectivity of the alkylative and protic glycosidations using all the DNA bases, A, G, C (cytosine) and T (thymine) bases. These results are summarized in Table 1. As expected by the Maxam–Gilbert DNA sequence protocol, the A base possessing the *N*-3 site, which was readily alkylated or protonated by several reagents,[4,5] was also effectively activated by both MeOTf and TfOH, as well as the G base, to give the glycosides **3** and **4** in high yield. In drastic contrast, the C and T bases were completely inactive under similar conditions. These results clearly indicated the high chemoselectivity of the glycosidation, that is, purine bases (A and G) worked as good functional groups of the glycosyl donors, while the pyrimidine

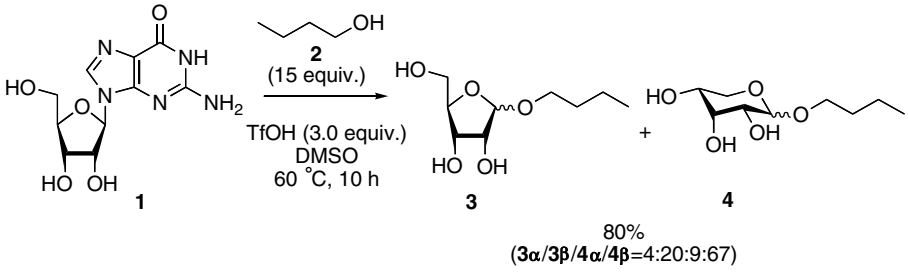
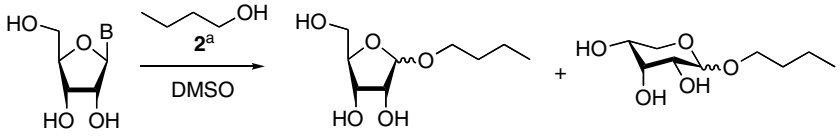


Figure 4. Synthesis of glycosides by the protic glycosidation of guanosine (**1**).

Table 1. Chemoselective glycosidations using DNA bases

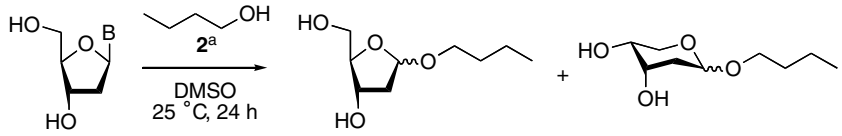


1: B=G (Guanine)
5: B=A (Adenine)
6: B=C (Cytosine)
7: B=T (Thymine)

Entry	Donor	Reagent/conditions	Yield (%)	Ratio (3α/3β/4α/4β)
1	**1**	A[b]	81	5:25:9:61
2	**1**	B[c]	80	4:20:9:67
3	**5**	A	81	7:23:10:60
4	**5**	B	94	4:20:10:66
5	**6**	A	—	—
6	**6**	B	—	—
7	**7**	A	—	—
8	**7**	B	—	—

[a] 15 equiv of **2** to the glycosyl donor was used.
[b] The reaction was carried out using 3 equiv of MeOTf at 80 °C for 5 h.
[c] The reaction was carried out using 2 equiv of TfOH at 60 °C for 10 h.

Table 2. Chemoselective glycosidations using DNA nucleosides



8: B=G (Guanine)
9: B=A (Adenine)
10: B=C (Cytosine)
11: B=T (Thymine)

12 **13**

Entry	Donor	Reagent/conditions	Yield (%)	Ratio (**12α/12β/13α/13β**)
1	**8**	A[b]	86	25:22:14:39
2	**8**	B[c]	84	13:10:15:62
3	**9**	A	89	40:38:7:15
4	**9**	B	93	13:10:16:61
5	**10**	A	—	—
6	**10**	B	—	—
7	**11**	A	—	—
8	**11**	B	—	—

[a] 15 equiv of **2** to the glycosyl donor was used.
[b] The reaction was carried out using 3 equiv of MeOTf.
[c] The reaction was carried out using 2 equiv of TfOH.

bases (C and T) had no such ability under the glycosidation conditions. Furthermore, we confirmed that the same chemoselectivity was observed when DNA nucleosides, **8**, **9**, **10** and **11**, all of which possess 2-deoxyribose, were employed as glycosyl donors (Table 2). Thus, the glycosidations of **8** and **9** with **2** smoothly proceeded at 25 °C to afford the glycosides **12** and **13** in high yield. In these cases, the glycosidations proceeded under milder conditions than those for the RNAs due to the higher reactivity of the 2-deoxy sugars.

Our attention then turned to the effect of the protecting groups of the glycosyl donor on the glycosidation reaction. Therefore, we next examined the glycosidations of the benzyl-protected glycosyl donor **14** and **2** using MeOTf or TfOH as the activating agent. We found that both glycosidations smoothly proceeded to give only the furanosides **15** in good yield with high β-stereoselectivity (Fig. 5). The high β-stereoselectivity must arise from the steric repulsion between the C2′ substituent of the ribose and the approaching alcohol. It was confirmed that the protection of the C5′ hydroxyl group of **1** prevented equilibrium between the produced furanosides **15** and the corresponding pyranosides. Furthermore, when the protected **14** was used as the glycosyl donor, large excess amounts of the alcohol **2** were not necessary for obtaining the high yield of **15**, and the secondary alcohol such as **16** was also applicable as the glycosyl acceptor to give the corresponding glycosides **17** in good yield, because the highly reactive hydroxyl group at the C5′ position of the glycosyl donor was protected with benzyl group. These results clearly indicated that the protection of the hydroxy groups of the glycosyl donor made it possible to produce only the corresponding alkyl β-furanoside in high yield.

Based on these results, we finally tested the conversion of a DNA oligomer into the chemoselectively modified DNA oligomer by this glycosidation method. We found

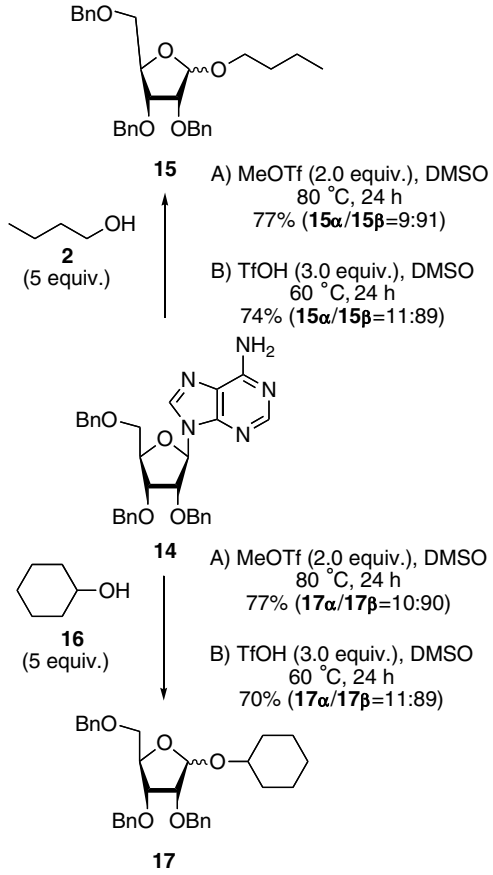


Figure 5. Synthesis of glycosides by the alkylative and protic glycosidations of protected adenosine **14**.

that the glycosidation of the DNA oligomer (5′-TA-3′) **18** and **2** using TfOH in DMSO smoothly proceeded to directly furnish the alkyl-substituted DNA oligomer **19** at the purine base, A, position. This result indicated that the phosphate linkage in the DNA oligomer is quite

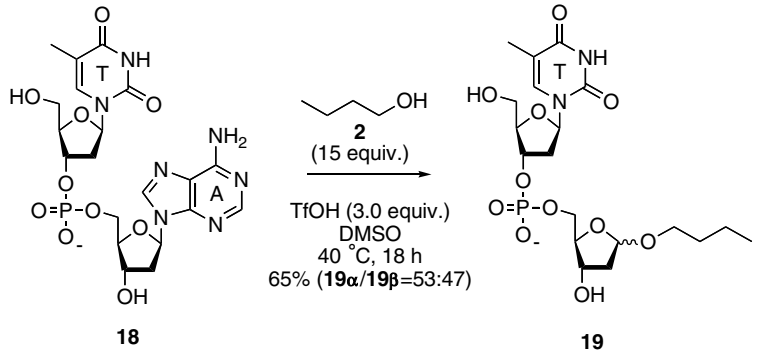


Figure 6. Synthesis of modified DNA oligomer **19** by the glycosidation of using DNA oligomer **18**.

stable under the glycosidation conditions, and worked well as the protecting group at the C5′ position of the 2-deoxyriboside in the DNA oligomer (Fig. 6).

In summary, we have demonstrated the one-step and chemoselective synthesis of alkyl glycosides and alkyl-substituted DNA oligomers by novel alkylative and protic glycosidations using DNA bases and DNA oligomers. Since DNA oligomers with any length and any sequence are easily available, the synthesis of many different types of modified DNA oligomers will be realized and will provide new possibilities in material science. These studies along this line are now in progress in our laboratories.

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